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The Population Genetics of the Endangered Scalloped Hammerhead Shark, Sphyrna lewini, Across its Eastern Pacific Range

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THE POPULATION GENETICS OF THE ENDANGERED SCALLOPED HAMMERHEAD SHARK, *SPHYRNA LEWINI*, ACROSS ITS EASTERN PACIFIC RANGE

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by
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ABSTRACT

I have characterized the population genetic structure, inferred the evolutionary processes shaping it, and estimated effective population size (N_e) using different contemporary and coalescent methods in the endangered scalloped hammerhead shark, *Sphyrna lewini*, throughout its Eastern Pacific (EP) range. I found significant genetic differentiation among seven coastal sites between Mexico and Ecuador using 15 microsatellite loci, and significant isolation by distance among samples of mtDNA control region haplotypes. While Bayesian statistical analyses and coalescent-based methods revealed low levels of ecological connectivity between most sampled sites (point estimates of Nm = 0.6 – 7.3), mismatch analyses showed that all populations experienced a relatively ancient expansion roughly 220,000 years ago (suggesting a common demographic history). Following this ancient expansion, EP *S. lewini* experienced steep declines in genetic diversity ($\Theta = 4N_e\mu$) and populations diverged within the last several centuries. Both decline and divergence happened concurrently, as 90% posterior probability densities of time since divergence overlap with those of time since decline. This overlap suggests a causal relationship between the two and both may be responsible for the genetic structure evident throughout the EP today. Population decline likely resulted in fewer migrants and lower ecological connectivity. Smaller, isolated populations then experienced a greater magnitude of genetic drift, ultimately driving their rapid diversification throughout the EP. The recent timing of these events and their overlap with historical fishing practices throughout this region highlight the evolutionary impact that overfishing can have on natural populations.

DEDICATION

This work is dedicated to Jerry Waldvogel. As a friend and mentor, he encouraged and inspired me to do more than just my research while a graduate student. I only wish we could toast my finishing with a dark ‘n’ stormy!

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CHAPTER ONE

NEW MICROSATELLITE LOCI FOR THE ENDANGERED SCALLOPED HAMMERHEAD SHARK, *SPHYRNA LEWINI*

Abstract

We isolated 15 microsatellite markers for the scalloped hammerhead shark, *Sphyrna lewini*. Loci were tested on 80 specimens of *S. lewini* from four Eastern Pacific samples. The number of alleles per locus range from six to 31 (mean = 14). Observed and expected levels of heterozygosity per locus range from 0.39 to 0.91 (mean = 0.70) and 0.54 to 0.90 (mean = 0.76), respectively. No pairs of loci were in gametic disequilibrium after Bonferroni correction of α. One locus showed significantly lower heterozygosity than expected under Hardy-Weinberg proportions in two populations, possibly caused by null alleles.

Primer Note

The scalloped hammerhead, *Sphyrna lewini*, is a circumtropical species (Compagno 1984) listed as 'endangered globally' on the IUCN Red List. Although landings of *S. lewini* have decreased in recent years (e.g. Vooren *et al.* 2005, Dudley & Simpfendorfer 2006, Martínez-Ortíz *et al.* 2007), the frequency of *S. lewini* and *Sphyrna zygaena* fins auctioned off in Hong Kong fish markets remains high, comprising nearly 5% of the total market fin weight (Clarke *et al.* 2006). Accordingly, data regarding stock structure, migration rates, and estimates of population size are needed to design effective conservation strategies. Species-specific microsatellite markers provide a means of

obtaining these data for threatened and endangered taxa. We have therefore isolated and characterized 15 microsatellite loci for *S. lewini* using library enrichment protocols of Clark and Brazeau (2005), modified from strategies developed by Kandpal *et al*. (1994).

The library was constructed using genomic DNA extracted from two juvenile sharks from Kaneohe Bay, Hawaii. DNA was digested using the *Sau* 3A enzyme, and fragments corresponding to 400-1500 bp were selected by cutting digested DNA out of a 3% agarose gel. *Sau* 3A linkers were ligated to size-selected DNA, and these fragments were enriched by PCR using the DNA Engine DYAD Peltier Thermal Cycler (MJ Research Inc.). The enriched DNA was hybridized to a biotin-labeled probe containing a CA-repeat sequence. Enrichment of these probe-targeted fragments was done using Vectrex® Avidin D matrix (Vector Laboratories). Non-specifically bound fragments were removed through a series of stringent washes, followed by elution and PCR amplification of CA-enriched fragments.

Fragments from the CA-enriched library were cloned by transforming *Escherichia coli* with product from the enriched library using the TOPO® TA Cloning Kit (Invitrogen Corporation). Colonies were submitted to the Clemson University Genomics Institute (CUGI) for sequencing on an ABI 3130 (Applied Biosystems), and subsequently screened for CA-repeat motifs using Sequencher (Gene Codes Corporation). Ninety pairs of primers were designed for sequences containing repeat regions using Web Primer (Stanford University).

Loci yielding amplification products of expected size on agarose gels were amplified using fluorescent dye-labeled primers. A three-primer protocol was used

(Hauswaldt & Glenn 2003; Toonen, pers. comm.) in which the following sequences were attached to reverse primers corresponding to dye-labeled tags incorporated into the PCR: 6-FAM-GGAAACAGCTATGACCAT, VIC-CAGTCGGGCGTCATCA, NED-ACCAACCTAGGAAACACAG, PET-GGCTAGGAAAGGTTAGTGGC. These loci were optimized on a larger sample of individuals. In general, PCR reactions were 12µl total volume, with 1X GoTaq buffer, 0.5µl forward primer, 0.05µl unlabeled reverse primer, 0.45µl dye-labeled reverse tag (primers at 5µM concentration), 0.125µl of 10% Triton X-100, 40µM dNTPs, 0.35U GoTaq polymerase (Promega), and 0.5µl DNA. Thermo-cycling profiles for each locus differed in annealing temperature (Table 1), but all started with denaturing at 95º (4 minutes), 30-40 cycles of 95º (1 minute), 55º -60º (1 minute), and 70º (1 minute), followed by a final extension at 70º (10 minutes).

Fluorescently-labeled products were run on an ABI 3130 capillary sequencer, using LIZ600 size standard (Applied Biosystems) and analyzed with GeneMapper® (Applied Biosystems). Loci that amplified consistently were further analyzed in MicroChecker (Van Oosterhout *et al.* 2004) to test for genotyping errors and aid in identification of possible null alleles. Arlequin (Schneider *et al*. 2000) was used to test for deviations from Hardy-Weinberg (HW) proportions and calculate observed and expected heterozygosities. GENEPOP (Raymond & Rousset 1995) was used to test for gametic-disequilibrium between all pairs of loci.

A total of 15 loci were characterized and found to be polymorphic among 80 individuals of *S. lewini* from four different Eastern Pacific samples. Data from these loci, including primer sequences, number of alleles, range of alleles, annealing temperature,

observed and expected heterozygosities, and p-values are listed in Table 1.1. All motifs were interrupted or imperfect CA-repeats. Sequences of each microsatellite can be viewed on GenBank (accession numbers in Table 1.1).

Seven loci (SLE013, SLE018, SLE027, SLE045, SLE053, SLE054, and SLE071) had significantly different values of observed and expected heterozygosity. However, departures from HW proportions at these loci were found in two of four populations at most, suggesting demographic factors may be responsible. Only one locus (SLE018) showed evidence of null alleles in two populations. No significant gametic-disequilibrium was detected across all pairs of loci after Bonferroni correction of α. We plan to use these markers to analyze populations of *S. lewini* at large, global scales and along smaller, continental margins.

Literature Cited

Clark G, D Brazeau (2005) Molecular Markers: Tools for Developing Enriched Microsatellite Libraries. The University of Florida, Gainesville.

Clarke SC, JE Magnussen, DL Abercrombie, MK McAllister, MS Shivji (2006) Identification of Shark Species Composition and Proportion in the Hong Kong Shark Fin Market Based on Molecular Genetics and Trade Records. *Conservation Biology* **20**, 201-211.

Compagno LJV (1984) FAO Species Catalogue. Vol. 4. Parts 1 & 2, Sharks of the world. *FAO Fisheries Synopsis*, p. 125.

Dudley S, C Simpfendorfer (2006) Population status of 14 shark species caught in the protective gillnets off KwaZulu-Natal beaches, South Africa, 1978-2003. *Marine and Freshwater Research* **57**, 225-240.

Kandpal RP, G Kandpal, SM Weissman (1994) Construction of libraries enriched for sequence repeats and jumping clones, and hybridization selection for region-specific markers. *Proceedings of the national Academy of Sciences* **91**, 88-92.

Martínez-Ortíz J, F Galván-Magaña, M Carrera-Fernández, D Mendoza-Intriago, C Estupiñán-Montaño, L Cedeño-Figueroa (2007) Abundancia estacional de Tiburones desembarcados en Manta – Ecuador, Pp. 9-27 in J Martínez-Ortíz and F Galván-Magaña (eds). Tiburones en el Ecuador: Casos de estudio. EPESPO – PMRC. Manta - Ecuador.

Raymond M, Rousset F (1995) GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *Journal of Heredity* **86**, 248-249

Schneider S, D Roessli, L Excoffier (2000) Arlequin: A software for population genetics data analysis. Ver 2.000. Genetics and Biometry Lab, Dept. of Anthropology, University of Geneva.

Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes* **4**, 535-538.

Vooren CM, S Klippel, AB Galina (2005) Biología e status conservação dos tubarão martelo *Sphyrna lewini* e *S. Zygaena,* Pp. 97-112 in CM Vooren and S Klippel (eds.) Ações para a conservação de tubarões e raias no sul do Brasil. Igaré, Porto Alegre.

Table 1.1: Primer sequences for new *S. lewini* microsatellite loci. T_a is annealing temperature. H_E and H_O are averaged expected and observed heterozygosities across all four Eastern Pacific samples, and p-values are given as the range across all four samples*.

Locus	Primer sequence	Access. no.	Alleles	Range (bp)	T*a*	HE	HO	p-value
SLE013	F: ATGTTTATGACCATACGTGCG R: TTGATTGGCATTCAGTGACC	FJ236873	10	302-350	60˚	0.67	0.55	0.05-0.91
SLE018	F: ACAGAAACAGAACGAGGGACA R: TGGGTTGGCATTGAACAGAA	FJ236878	6	208-258	60˚	0.65	0.40	0.00-0.90
SLE025	F: CTCAGGCTAGTTGCACAGAAA R: TCAACTCCCCACAATCCCAT	FJ236874	31	234-398	57˚	0.90	0.91	0.96-0.10
SLE027	F: GAGACCAGCCAAAGGAAAAA R: ATGCCATATTCATCCAGGCAC	FJ236879	13	420-459	60˚	0.70	0.61	0.03-0.84
SLE028	F: TTTGGAGACATTGCAGAAAG R: CACTTGGGACTACACACACTG	FJ236875	25	226-281	55˚	0.84	0.88	0.06-0.83
SLE033	F: TTGGTCAATGTCCTCTTGCA R: CCCATGCTGTTTTGTTCTTTG	FJ236876	11	261-299	57˚	0.79	0.80	0.16-0.60
SLE038	F: AGCCTACTTCTGCCACATTTT R: AATCAAAGTTCCTGCAGTCCT	FJ236877	14	419-475	60˚	0.80	0.73	0.06-0.63
SLE045	F: AGGATGGGATTCAGTGACAGA R: AATAAGCTCAAAGGGCTGGA	FJ236880	5	403-411	60˚	0.66	0.57	0.01-0.96
SLE053	F: AAGTCAAAAGCTGTGTGCGA R: ATTCCCCACATACATTCCCCA	FJ236881	21	407-457	57˚	0.84	0.79	0.06-0.79
SLE054	F: CTGACACTGCCAATTTGCAT R: CCAACTGGAGTTGTCAATCCA	FJ236882	9	186-206	60˚	0.54	0.39	0.06-0.78
SLE071	F: TCAGACGGTGGTACGTACACA R: TGACCCTTTTGGATTGAAGGA	FJ236883	13	236-285	60˚	0.75	0.58	0.01-0.54
SLE077	F: TTCCCTCTCAGAGTGACATTG R: CCTTTCCTCCATACACAAACA	FJ236884	29	218-317	55˚	0.90	0.91	0.53-0.78
SLE081	F: ATGTTCATCATCCGAGACAGG R: CCAAACACACGTATCTGCACCCA	FJ236885	8	384-402	60˚	0.80	0.81	0.62-0.99
SLE086	F: TACAGACAGATTTCAGTGTGT R: ACGAATACGCATTCATACAC	FJ236886	6	350-361	60˚	0.71	0.67	0.18-0.62
SLE089	F: TTACCACAGTTTGTGTGGGTG R: AAGTTTCAGTGTCAGTGTGC	FJ236887	13	172-204	60˚	0.85	0.91	0.25-0.85

CHAPTER TWO

DECLINE AND DIVERGENCE: EVOLUTIONARY PROCESSES CAUSING POPULATION STRUCTURE IN THE SCALLOPED HAMMERHEAD SHARK, *SPHYRNA LEWINI*

Abstract

We have characterized the population genetic structure, and inferred the evolutionary and demographic processes shaping it, in the endangered scalloped hammerhead shark, *Sphyrna lewini*, throughout its Eastern Pacific (EP) range. Using 15 microsatellites, we found significant structure among seven coastal sites between Mexico and Ecuador, and significant isolation-by-distance among a 548bp portion of mtDNA control region. Bayesian statistics and coalescent-based methods showed low levels of ecological connectivity between most sampled sites (point estimates of Nm = 0.6 – 7.3), yet all populations experienced a relatively ancient expansion ~220,000 years ago (suggesting a common demographic history). However, populations diverged more recently, within the past few centuries. Coincident with this divergence were steep declines in genetic diversity ($\Theta = 4N_e\mu$), as 90% posterior probability densities of time since divergence overlap with those of time since decline. This overlap suggests the decline and divergence are causally related and responsible for the genetic structure evident throughout the EP today. Population decline could have led to fewer migrants and lower connectivity. Smaller, isolated populations experienced a greater magnitude of genetic drift, leading to their rapid diversification throughout the EP. The recent timing of these events highlights the evolutionary impact that overfishing can have on natural populations.

Introduction

Although species with far reaching distributions and perceived high dispersal potential have often been assumed to consist of large, genetically uniform populations, a growing number of studies have demonstrated unexpected population differentiation at a variety of spatial scales (e.g., Waits et al. 2000, Abbott & Double 2003, Fredsted et al. 2005). For species in the sea, unexpected patterns of population differentiation, sometimes over relatively small distances, have been described in species with planktonic larvae (e.g., Todd et al. 1998**,** Barber et al. 2000**,** Taylor & Hellberg 2003**,** Perrin et al. 2004, Sotka et al. 2005, Marko & Barr 2007) as well as large vertebrates capable of sustained swimming over large distances (e.g., Baker et al. 1986, Morreale et al. 1996, Rooker et al. 2007).

As more detailed descriptions of genetic structure have accumulated, the focus of marine population geneticists has gradually shifted from characterizing patterns of genetic differentiation to disentangling the population genetic forces that have together acted to create the observed patterns. For the most part, marine phylogeographers have interpreted significant genetic differentiation as evidence of localized limits on gene flow, particularly when sharp genetic breaks are associated with oceanographic discontinuities or other potential barriers to dispersal (e.g., Palumbi & Warner 2003, Sotka et al. 2005, Marko & Barr 2007, Pelc et al. 2009). Although reduced gene flow could be primarily responsible for spatial patterns of genetic variation in many situations, a full understanding of the history and evolutionary development of spatial differentiation requires consideration of the combined effects of the entire suite of relevant interacting

evolutionary forces over time, including mutation, gene flow, and genetic drift. Determining the cause for any particular 'snapshot' of spatial genetic structure observed today therefore requires distinguishing the relative importance of evolutionary and demographic mechanisms involved over time. By considering the temporal component to patterns of genetic differentiation, a variety of alternative demographic histories - each of which can potentially paint similar pictures of genetic structure - may be distinguished, such as ancient population divergence combined with moderate levels of gene flow (e.g. Buhay & Crandall 2005) versus much more recent population isolation combined with more restricted gene flow (e.g. Niemiller et al. 2008).

The scalloped hammerhead shark, *Sphyrna lewini*, is an example of a large, highly-mobile marine predator for which population structure and, more importantly, the processes shaping it are poorly understood. This circumtropical species, found along continental margins and oceanic islands (Compagno 1984) forms large, conspicuous aggregations, particularly in the tropical Eastern Pacific (EP) Ocean (Torres-Huerta 1999, Martínez-Ortíz et al. 2007, Zanella 2008). Like other large marine vertebrates, tagging studies have revealed long-distance dispersal of individuals between volcanic islands, seamounts, and embayments, but also provide evidence of some site fidelity to natal pupping grounds (Klimley 1981, 1985; Klimley & Nelson 1981, Klimley et al. 1988, 1993; Kohler et al. 1998). While these observations have led to the expectation of population differentiation between distinct pupping grounds, genetic data can potentially be used to test this prediction and further determine whether movements from tagging

studies represent a historical, persistent pattern of limited gene flow or a more recent reduction in connectivity.

Previous genetic analyses of *S. lewini* have yielded partially resolved patterns of genetic differentiation on different spatial scales. Though mitochondrial DNA (mtDNA) control region sequences showed a consistent pattern of significant differentiation across ocean basins, no significant differentiation was evident on smaller spatial scales, along individual coastlines (Duncan et al. 2006). While patterns of gene flow could vary on different spatial scales, the apparent genetic homogeneity along some coastlines, such as the eastern Pacific, could also reflect characteristically low mtDNA diversity in sharks (Martin et al. 1992) combined with recent population separations and insufficient sampling of markers and individuals. To better understand intraregional patterns of population differentiation and the evolutionary processes governing them, we have used a combination of mtDNA sequences and 15 nuclear microsatellite loci to re-visit patterns of genetic differentiation in *S. lewini*. Despite the expectation that nuclear genes will be "lagging indicators" of patterns of population structure (Zink & Barrowclough 2008), inferences about population genetic processes cannot be reliably inferred without a multilocus approach (Edwards & Bensch 2009). With this multi-locus approach, our study addresses three questions: 1) Are individual aggregations of the apparently philopatric species *S. lewini* genetically distinct along the EP coast? 2) What population genetic processes have been most important in generating these patterns? 3) Over what timescales have these processes been acting? Our inferences about demographic history

reveal an extremely recent population separation coincident with large reductions in effective population size in this globally endangered species (IUCN 2007).

Methods

Sampling, DNA Extraction, Sequencing, and Genotyping Procedures

We collected 396 tissue samples from fishers at six Eastern Pacific sites in Mexico, Costa Rica, Panama, and Ecuador between 2006 and 2008 (Figure 2.1, Table 2.1). Samples were stored in 90% ethanol and genomic DNA was isolated with proteinase K tissue digestion in 2X CTAB, followed by two chloroform:isoamyl alcohol (24:1) extractions and precipitation with ethanol. DNA was dried, re-suspended in 50µL water, and frozen.

We amplified and scored 15 microsatellite loci from 387 individuals. Thirteen were developed for *S. lewini* [see Nance et al. (2009) for PCR conditions], and two (Cli-12 and Cli-100) were developed for the blacktip shark (Keeny & Heist. 2003). All PCR reactions were conducted using a DNA Engine DYAD Peltier Thermal Cycler (MJ Research, Inc.) and visualized on an ABI 3130 (Applied Biosystems, Inc.) automated sequencer at the Clemson University Genomics Institute (CUGI). Individual genotypes were scored with GeneMapper v. 3.7 (Applied Biosystems, Inc.).

A 548 bp fragment of the mtDNA control region was sequenced from 126 individuals. We initially amplified a ~1200 bp fragment using the Pro-L and SLcr-H primers from Duncan et al. (2006) with the following cycling temperature profile: 95 ºC for 4 minutes, 40 cycles of 95 ºC for 1 minute, 57 ºC for 1 minute, slow ramp (1 ºC/s) to

72 ºC for 1 minute, 30 seconds, followed by an extension at 72 ºC for 10 minutes. Each PCR reaction contained 1X GoTaq buffer, 0.16µM Pro-L primer, 0.16µM SLcr-H primer, 0.1% Triton X-100, 1.25mM dNTPs, 0.7U GoTaq polymerase (Promega), and 0.5µl genomic DNA, in a total volume of 25 µl. Because Duncan et al. (2006) found informative sites only at one end of the fragment, we only sequenced with the Pro-L primer. However, any chromatograms that contained ambiguous base calls were also sequenced in the opposite direction with the SLcr-H primer. Sequencing reactions were visualized on an ABI 3130 automated sequencer, chromatograms edited with Sequencher v.4.2.2 (Gene Codes Corp.), and aligned using Clustal-X v.1.81 (Thompson et al. 1998). All sequences were from individuals sampled in 2007 with the exception of those from two locations in Panama (CEB and GPA).

Microsatellite and MtDNA Diversity

Loci were checked for evidence of nulls using Micro-Checker v. 2.2.3 (Van Oosterhout et al. 2004), and tested for deviations from Hardy-Weinberg equilibrium and linkage disequilibrium using Arlequin v. 3.11 (Excoffier et al. 2005). For the mtDNA, we used two statistics to detect departures from drift-mutation equilibrium. Fu's F_s (Fu 1996) was calculated in Arlequin, and significance was evaluated by comparison to 10,000 simulations. Fu and Li's D^* (Fu & Li 1993) was calculated in DNAsp v. 4.90 (Rozas et al. 2003). Each neutrality statistic was estimated for each individual sample.

Genetic Structure

Samples were collected between 2006 and 2008, so before examining patterns of spatial genetic differentiation with the microsatellite data, we first tested for differences between years to avoid bias from potential temporal structure. We estimated F_{ST} between samples from the same location in different years, and then estimated F_{ST} among sampling sites with a locus-by-locus Analysis of Molecular Variance (AMOVA) in Arlequin. Confidence intervals for F_{ST} were generated by bootstrapping over loci (20,000 replicates). We also estimated R_{ST} to have an analogue to Φ_{ST} estimates based on mtDNA.

With the mtDNA, we created a haplotype network with TCS v.1.21 (Clement et al. 2000) using statistical parsimony (Templeton et al. 1992). We then used MODELTEST v. 3.8 (Posada & Crandall 1998) to identify the best-fitting model of nucleotide substitution. Using the best-fitting model available in Arlequin [Tajima & Nei (1984)], we then calculated F_{ST} and Φ_{ST} with AMOVA.

Demographic Analyses

We conducted mismatch analyses in Arlequin with the mtDNA data by comparing the distribution of observed haplotype differences to that expected under a model of sudden expansion. To determine how well the sudden expansion model fit our data, we calculated Harpending's raggedness index, r (Rogers & Harpending 1992), and assessed significance of r with 1000 parametric bootstrap replicates. For distributions not deviating significantly from the sudden expansion model, we estimated the intrapopulation coalescence time, or time since the start of expansion, from statistic τ

using the formula $\tau = 2\mu t$, where t is the number of years since expansion and μ is the per locus per year mutation rate. Confidence intervals for τ were estimated by 1000 parametric bootstrap replicates in Arlequin.

We calculated the M-ratio (the mean ratio [M] of the number of alleles to range in allele size for all microsatellite loci) of Garza & Williamson (2001) to test for evidence of a recent population bottleneck in each EP sample. A rapid loss of rare alleles in a bottlenecked population causes this ratio to decrease. The empirical value of M, calculated using the software *M_P_val* (Garza & Williamson 2001) was compared to a simulated equilibrium distribution of M based on the two-phase model of microsatellite mutation. This value, M_C, was calculated by running 10,000 replicates in *critical_M* (Garza & Williamson 2001). We analyzed our data using two different values for p_s, the mean percentage of mutations that follow the single-step mutation model, and Δ_g, the mean size of larger mutations. As suggested by Garza & Williamson (2001), we first used $p_s = 0.88$ and $\Delta_g = 2.8$, and then used more conservative values $p_s = 0.90$ and $\Delta_g = 3.5$. However, the values of M for our populations were equal for both values for p_s and Δ_g. Therefore, we only showed M-ratios calculated for the latter of the two parameters. To test for the significance, we used a range of values for pre-bottleneck population Θ (0.01, 0.1, 1.0, 10.0), which would yield a pre-bottleneck N_e of 250, 2500, 25,000, and 250,000, respectively, using equation $\theta = 4N_e\mu$. Here, we chose a microsatellite mutation rate (μ) of 1×10^{-5} because this is the slower end of the range estimated in mammals (e.g. Dallas 1992, Weber & Wong 1993, Ellengren 1995, Yue et al. 2002) and mitochondrial and nuclear markers mutate roughly an order of magnitude slower in sharks than in

mammals (Martin et al. 1992, Martin 1999). We used values of $p_s = 0.90$ and $\Delta_g = 3.5$ to calculate M_C as recommended by Garza & Williamson (2001).

We estimated current and ancestral N_e with MSVAR v. 1.3 (Beaumont 1999) which uses Markov Chain Monte Carlo (MCMC) simulations of the mutation-coalescent history for present day microsatellite genotypes in a sample. Random samples are drawn from the Bayesian posterior distributions of demographic and mutational parameters and their likelihoods are calculated (Beaumont 1999). We estimated the posterior distribution of the parameters N_0 (current population size), N_1 (ancestral population size), μ (mean mutation rate of all loci), and t (time since population size change) for each population. We analyzed all samples but GPA, due to its small sample size. We set mean generation time to 22 years [estimated using Felsenstein's (1971) equation and data from Cortés et al. (2009), Piercy et al. (2007), and Nance & Sloop (unpublished data)], and varied priors for each locus for N_0, N_1, μ, and t, as suggested by the author. Prior values were updated throughout the analysis, and modeled an exponential change in population size. Each run was 200 million steps, with a burn-in of 10,000 steps and output every 10,000 steps. We used TRACER v. 1.4.1 (Rambaut & Drummond 2007) to graph posterior distributions of N_0, N_1, μ, and t, and to calculate the 95% mean probability densities of each parameter.

Population Divergence Times and Migration Rates

We estimated migration rates (m_1 and m_2), time since population divergence (t), and genetic diversities (Θ_1, Θ_2, and ancestral Θ_A) for all pairs of adjacent samples (except GPA due to small sample size) using the program IMa (Hey & Neilsen 2007) on

the CBSU computing clusters at Cornell University. This coalescent-based program simulates gene genealogies using MCMC sampling methods. The "isolation with migration" model in IMa does not assume populations are in drift, migration, and mutation equilibrium, making it more appropriate for recently diverged populations that share haplotypes and alleles due to both recent gene flow and ancestral polymorphism.

We started with an analysis in "MCMC Mode" using the full complement of model parameters (i.e., $\Theta_1 \neq \Theta_2 \neq \Theta_A$, and $m_1 \neq m_2$), with broad priors for all, reducing them accordingly in repeated runs. Once several replicates converged on the same answer, we ran the saved genealogies from three separate M-Mode runs in a new analysis using the nested models option in "Load Trees Mode" to determine if the fully parameterized IMa model was a significantly better fit to the data than a series of simpler models with fewer parameters. If a simpler model was a significantly better fit to the data, we reanalyzed with that model. In the final runs for each analysis, we recorded the maximum likelihood estimate (MLE) for each parameter and 90% highest posterior density interval or HPD. From these values, we converted migration parameters m_1 and m_2 into the number of migrants per generation, $Nm = (\Theta m)/4$.

To convert divergence times from IMa scaled by mutation (t/μ) into units of years, the mutation rate of at least one locus must be known. Given that microsatellite mutation rates are unknown for sharks and can vary by an order of magnitude within individual species (Bulut et al. 2009), we used only mtDNA mutation rates calculated specifically for *S. lewini* and allowed IMa to infer mutation rate scalars for the microsatellite loci (Hey 2007). As an upper bound on mtDNA control region mutation

we used a previously published rate of 4.00 x 10^{-9} subs/year based on the assumption that Western Atlantic and Eastern Pacific populations of *S. lewini* were separated three million years ago by the Isthmus of Panama (Duncan et al. 2006). For a lower bound, we followed Martin et al. (1992) by using the first appearance of *Sphyrna* in the fossil record 20-23 million years ago (Cappetta 1987) to calculate a mutation rate (3.21 x 10^{-9} subs/year) from the control region sequence divergence between *S. lewini* and a previously published sequence from *S. tiburo* (GenBank accession #DQ168923, Quattro et al. 2006). Sequence divergences were calculated with PAUP v.4.0 (Swofford 2002) using the best-fitting substitution model (Hasegawa et al. 1985) selected with MODELTEST. Neither the Isthmus-based nor the fossil-based rate is likely accurate: Isthmus-based calibrations tend to over-estimate mutation rates (Marko 2002; Lessios 2008) whereas our fossil-based rate almost certainly underestimates the rate given that the oldest fossil *Sphyrna* are neither *S. lewini* nor *S. tiburo* (Cappetta 1987). However, the fossil calibration and the Isthmus calibration together provide a very conservative range within which we estimated population divergence times.

Results

Microsatellite and MtDNA Diversity

Three microsatellite loci in two of ten sampled populations deviated from Hardy-Weinberg equilibrium (HWE) expectations after sequential Bonferroni correction of α (Rice 1989); another locus deviated from HWE in one of ten samples (also see Appendix A). After sequential Bonferroni correction, two loci (Cli-12 and Cli-100) were in linkage

disequilibrium in two of ten samples. Micro-Checker showed five loci had no nulls in any of the samples, and ten had potential nulls in one or two samples.

We found seven mtDNA control region haplotypes. Haplotypes A and B were common to all locations (see Figure 2.2) and C through D were found in one to two locations (Table 2.1). Fu's F_s was positive for each sample, though none were significant (Table 2.1). Fu and Li's D^* was negative for three samples: TAR, SCA and CEB, though none were significant (Table 2.1).

Genetic Structure

At individual localities, only microsatellite estimates of F_{ST} from LAP, MAZ, and TAR were significantly differentiated between years (Table 2.2). Therefore, we only combined sample years from Santa Catalina and Manta and repeated spatial analyses by substituting each temporally distinct site, essentially treating them as separate samples (Tables 2.3 and 2.4). Global AMOVAs testing for spatial differentiation were highly statistically significant and within the same order of magnitude, regardless of sampling year we substituted (we only show F_{ST} values in Table 3, as all R_{ST} values were insignificant). We only show results from the 2007/2008 samples (omitting results with LAP and MAZ 2006) because all F_{ST} values using different years were within 0.001 of each other and all had p-values < 0.001.

Pairwise AMOVAs using microsatellite data showed significant genetic differences between most pairs of samples (we only show F_{ST} values in Table 2.4, as all R_{ST} values were non-significant). A Mantel test showed weak but marginally significant

correlation between genetic (F_{ST}) and geographic distance between sites (r = 0.302, p = 0.063). R_{ST} and geographic distance were correlated significantly (r = 0.422, p = 0.032).

For the mtDNA sequences, neither F_{ST} nor Φ_{ST} across all sites were statistically significant (only Φ_{ST} values shown in Table 2.3). No pairwise F_{ST} estimates were significant, however, pairwise estimates of Φ_{ST} showed significant differentiation between one central Panama sample (SCA) and both Mexico samples (SCA-LAP Φ_{ST} = 0.17, p = 0.03 and SCA-MAZ Φ_{ST} = 0.21, p = 0.01). The Mantel test, however, showed a significant correlation between Φ_{ST} and geographic distance (r = 0.523, p = 0.039), though no significant correlation was detected with the frequencies of haplotypes (F_{ST}).

Demographic Analyses

Mismatch distributions for mtDNA showed evidence of relatively ancient demographic expansions across all populations. For the mtDNA sequences, the model of sudden demographic expansion was only rejected for the southernmost population, MAN. For all populations, the modal number of nucleotide differences between haplotypes peaked between zero and one (Figure 2.3), indicating relatively recent expansions. After conversion with both mtDNA mutation rates, point estimates of time since expansion among all populations (excluding MAN) were between 136,530 and 255,114 years ago (Table 2.5).

In contrast to the mtDNA sequences, the microsatellite data exhibited evidence of more recent declines in population size. The ratio of the number of alleles to the range in allele size (M) for each population was lower than M_C, the critical value from 10,000

equilibrium replicates, for each value of Θ (Figure 2.4). Likewise, MSVAR analyses indicated current N_e was at least two orders of magnitude smaller than historic N_e and the onset of decline was between 3600 and 16,700 years ago (Table 2.6). Results from IMa also showed that $\Theta_A = 4N_e\mu$ was larger than Θ of current populations (Table 2.7).

Population Divergence Times and Migration

For each comparison of adjacent samples, each simpler demographic model in IMa was rejected in favor of the fully parameterized model (likelihood ratio tests not shown). Estimates *t* for all pairs of populations were significantly greater than zero, given that the posterior probability distributions drop to zero as *t* approaches zero for all population pairs. MLEs of t for pairwise population comparisons were all relatively recent: 98 - 1636 years ago (fossil calibration) and 77 - 1293 years ago (Isthmus calibration) (Table 2.7, Figure 2.5). The exception was an MLE of t = 38,090 years (fossil calibration) and 30,089 years (Isthmus calibration) between TAR and SCA (Table 2.7).

Maximum likelihood estimates (MLEs) of Nm (the number of migrants per generation) between all adjacent population pairs were between 0.4 and 7.3, estimates similar to those obtained from calculations based on F_{ST}. The exception was 243.1 migrants per generation between the two adjacent central Panama localities (SCA and CEB), separated by only 40 km (Figure 2.6; only adjacent population results shown).

Discussion

Our survey and analysis of patterns of mitochondrial and nuclear diversity in the scalloped hammerhead shark, *S. lewini*, indicated that populations in the EP are genetically distinct. Global AMOVAs based on data from 15 hypervariable microsatellite loci showed significant genetic differentiation, most pairwise F_{ST} values were significant, and estimates of Φ_{ST} from mtDNA and R_{ST} from microsatellite data showed significant isolation by distance (IBD). Simulation studies have shown that biologically meaningful patterns of IBD may be evident in genetic data even when rates of gene flow are high and levels of genetic differentiation are correspondingly low. Therefore, a significant IBD pattern may be a better indicator of limited exchange among populations than individual estimates of differentiation (Palumbi 2003).

Although many marine species show similarly subtle, yet statistically significant, patterns of population differentiation, two unanticipated results emerged from our analyses. First, genetic structure evolved only very recently, with MLEs for divergence time (t) between 77 and 1636 years ago, a range of dates including conversion of *t* into units of years with our conservative fossil-calibrated rate. The notable exception to this recent divergence was the estimate the TAR and SCA split, which appeared to have a much deeper divergence time of 38,090 (fossil μ) or 15,044 (Isthmus μ) years ago. However, this outlier estimate may have been due to a much higher IMa mutation rate scalar for this population pair (roughly indicating more variation in mtDNA relative to the other nuclear loci), and rate scalars are used to calculate time in years. Therefore, a higher scalar for a particular population pair will yield a deeper divergence time. For the other estimates, posterior distributions for t had strong peaks with probabilities dropping

to zero as t approaches zero, indicating our estimates of t, though close to, were significantly greater than zero.

The second surprising result was that nearly all population separations were associated with substantial decreases in genetic diversity (1-3 orders of magnitude in most cases when compared to ancestral Θ), reflecting large reductions in effective population size. The exception to this pattern was Cebaco Island, which had a current Θ only five times smaller than ancestral Θ, but only in the pairwise analysis with Santa Catalina. This larger, current estimate of Θ_{CEB}, however, was not apparent when Cebaco Island was analyzed with Tarcoles and Manta, and likely is not representative of the true level of genetic diversity (Θ). Although the onset of the decline in each population, as estimated with MSVAR, was older than the MLEs of t from IMa, they do overlap with the 90% HPDs for t across all populations.

This coincident, recent decline and divergence across all populations suggests these two demographic events are causally related and may be responsible for the genetic structure observed today. Steep population declines would result in fewer migrants, and lower connectivity, allowing interpopulation differences to evolve. Smaller, isolated populations would experience a greater magnitude of genetic drift, and this may have been the main evolutionary process causing diversification of EP *S. lewini* populations.

The next obvious question regarding the demographic history of *S. lewini* is *why* populations declined. Recently, much attention has focused on the effects of prehistoric fishing by indigenous peoples, showing they had already substantially reduced marine populations and altered ecosystems long before European contact (e.g. Jackson et al.

2001, Pinnegar & Engelhard 2008). For example, archaeological remains from 14 sites across the Pacific coasts of Costa Rica, Panama, and Ecuador suggest that fishers were catching epipelagic fish using primitive drift nets and watercrafts as early as 6000 to 1800 years ago (Cooke 1992), and given 3-5% of middens in this region are comprised of shark remains, fishers were most certainly catching them (Richard Cooke, *pers. comm*.). Given the propensity of *S. lewini* to utilize such shallow and protected nearshore embayments for both reproduction and maturation of juveniles (e.g. Clarke 1971, Compagno 1984, Klimley 1987, Castro 1993, Stevens & Lyle 1989), a concentration of pre-Colombian fishing practices in these environments could have significantly reduced the number of immature *S. lewini* long before 20th century long-liners were fishing in the pelagic realm of this semi-pelagic shark. Removing juveniles that have yet to replace themselves in a population will surely reduce N_e, particularly for sharks characterized by relatively low fecundity and late age at maturity (Musick et al. 2000).

A relevant caveat to the timing of population decline, and human activity being the ultimate cause of it, is that our demographic estimates based on the mutation rate, μ, might be wrong. If the control region mutation rates that we have used are too fast, then our estimates of divergence time will be biased downwards. However, we likely underestimated the mtDNA mutation rates for two reasons. First, our fossil-calibration based on the first appearance of *Sphyrna* roughly 21 mya is likely too slow, even for an estimate of the substitution rate. Although the more recent geologic (vicariant) calibration across the Isthmus of Panama at 3 mya may overestimate the substitution rate,

the demographic events we are concerned with dating are so recent, an instantaneous mutation rate is necessary.

Nonlinearity of the mtDNA molecular clock may arise in part due to purifying selection removing slightly deleterious mutations over hundreds of thousands of years, while such mutations are more numerous in the recent history of lineages (e.g. Howell et al. 2003, Ho et al. 2005, Endicott et al. 2009). The result is an instantaneous mutation rate which is faster than the allelic fixation, or substitution rate, leading to an underestimate of μ when the calibration point is old (> 50,000 yrs; Henn et al. 2009). Therefore, both of our calibration points yield a rate more reflective of the substitution rather than the mutation rate in *S. lewini*, rendering demographic estimates of time since divergence too old. Taking the time-dependency of a molecular clock and the decay curve characterized for the human mtDNA mutation rate into account, our most recent calibration point of 3 mya may underestimate the mutation rate by as much as 10-fold (Howell et al. 2003, Henn et al. 2009). Demographic parameters adjusted for a five to 10-fold faster mutation rate would place most divergence times within the last 300 years, suggesting more recent fishing practices caused the decline.

A second caveat for consideration is that microsatellite loci which violate the single-step mutation (SSM) model will lead to overestimates of demographic parameters like N_e and time since expansion or decline (Gonser et al. 2000). These violations may be likely in our data because most of our microsatellite loci probably do not conform to the SSM due to imperfect repeats and point mutations. Considering this, our estimates of time since decline and divergence are most likely biased upwards and it is therefore

reasonable to assume the onset of these two demographic events overlaps with an increase in historic fishing practices within the last few hundred years.

Gene Flow and Connectivity

Point estimates of migrants per generation (Nm) were low when considered from a demographic perspective. Both N_e based on IMa estimates of Θ and estimates from MSVAR reveal effective population sizes in the hundreds (see Figure 2.6), rendering less than 10 migrants per generation (equivalent to less than one migrant per year) ecologically insignificant (Vucetich & Waite 2000). While these point estimates of Nm were low on demographic scales, we cannot exclude the possibility that migration rates may be larger due to their corresponding large confidence intervals in IMa. However, because IMa estimates were only slightly less than estimates based on F_{ST} calculations, which tend to overestimate Nm when using sample sizes and numbers of loci similar to that in our study (Gaggiotti et al. 1999), it is likely that the point estimates rather than the upper 90% boundary of the posterior density, reflect the actual rate of migration.

When interpreting our migration results, we must also consider our sampling scale and scheme. Most of our samples came from sharks caught by artisanal fishermen close to shore. Therefore, we likely missed populations potentially exchanging migrants with ours. Simulations have shown a third, unsampled population exchanging migrants with one of two populations considered in an IMa analysis will increase estimates of migration into that population (Strasburg & Rieseberg 2009). Migrants from a third, unsampled population may also cause an upward bias in estimates of Θ_A and divergence time

(Strasburg & Rieseberg 2009). However, given these kinds of effects are minimal when migration rates are demographically low (Beerli 2004), it is unlikely that gene flow from unsampled populations has biased our estimates of demographic parameters enough to account for the large difference we observed between current and ancestral Θ.

Demographic History

Estimates of τ from mtDNA suggest a shared demographic history for EP populations of *S. lewini*, with all populations showing evidence of a relatively ancient demographic expansion roughly 185,260 - 259,538 years ago, long before the population separation times inferred from IMa. In contrast to this ancient expansion, more recent population declines roughly 3600 – 16,700 years ago based on microsatellite data (Table 2.6) overlap with the 90% HPDs of IMa divergence times (Table 7). Evidence for recent population declines were also supported by estimates of current and ancestral Θ from our IMa analyses (Table 2.7), and were evident in M-ratio values across all populations (Figure 2.4).

Overall, these data speak most clearly to the evolutionary impacts of human harvesting of natural populations. The timing of population decline and divergence coincides with historic fishing practices in this region, particularly when potential biases associated with the mutation rates and demographic parameters are considered. The decline in population size is not surprising, as IUCN Red Listing (IUCN 2007) and a recent CITES proposal demonstrate the recognition of decline in this species prior to our analyses. What is surprising, however, is that populations are now sufficiently small for

genetic drift, rather than long isolation time or extremely reduced gene flow, to be the primary evolutionary process driving recent diversification of EP populations that were previously not differentiated.

Literature Cited

Abbott, C. L. and M. C. Double. 2003. Genetic structure, conservation genetics and evidence of speciation by range expansion in shy and white-capped albatrosses. Mol. Ecol. 12:2953-2962.

Baker, C. S., L. M. Herman, A. Perry, W. S. Lawton, J. M. Straley, A. A. Wolman, G. D. Kaufman,H. E. Winn, J. D. Hall, J. M. Reinke, J. Östman . 1986. Migratory movement and population structure of humpback whales (*Megaptera novaeangliae*) in the central and eastern North Pacific. Mar. Ecol. Prog. Ser. 31:105-119.

Beaumont, M. A. 1999. Detecting population expansion and decline using microsatellites. Genetics 153:2013-2029.

Beerli, P. 2004. Effect of unsampled populations on the estimation of population sizes and migration rates between sampled populations. Mol. Ecol. 13:827-836.

Bizzarro, J. J., W. D. Smith, J. F. Márquez-Ferías, J. Tyminski, and R. E. Hueter. 2009. Temporal variation in the artisanal elasmobranch fishery of Sonora, Mexico. Fish. Res. 97:103-117.

Buhay, J. E., and K. A. Crandall. 2005. Subterranean phylogeography of freshwater crayfishes shows extensive gene flow and surprisingly large population sizes. Mol. Ecol. 14:4259-4273.

Bulut, Z. C. R. McCormick, D. Gopurenko, R. N. Williams, D. H. Bos, and J. A. DeWoody. 2009. Microsatellite mutation rates in the eastern tiger salamander (Ambystoma tigrinium tigrinium) differ 10-fold across loci. Genetica 136:501-504.

Cappetta, H. 1987. Chondrichthes II. Mesozoic and Cenozoic Elasmobranchii; vol. 3B. Handbook Paleoichthyology. Fisher. Stuttgart.

Castro, J. I. 1993. The shark nursery of Bulls Bay, South Carolina, with a review of the shark nurseries of the southeastern coast of the United States. Environ. Biol. Fish. 38:37-48.

Chapuis, M. P., and A. Estoup. 2007. Microsatellite null alleles and estimation of population differentiation. Mol. Biol. Evol. 24:621-631.

Clarke, T. A. 1971. The ecology of the scalloped hammerhead shark, *Sphyrna lewini*, in Hawaii. Pac. Sci. 25:133-144.

Clement, M. D., D. Posada, and K. A. Crandall. 2000. TCS: a computer program to estimate gene geneologies. Mol. Ecol. 9:1657-1660.

Compagno, L. J. V. 1984. FAO Species catalogue. vol. 4, parts 1 & 2, Sharks of the World. FAO Fisheries Synopsis, p. 125.

Cooke, R. 1992. Prehistoric nearshore and littoral fishing in the eastern Tropical Pacific: An ichthyological evaluation. J. World Prehist. 6:1-49

Cortés , E., F. Arocha, L. Beerkircher, F. Carvalho, A. Domingo, M. Heupel, H. Holtzhausen, M. Santos, M. Ribera, and C. Simpfendorfer. 2009. Ecological risk assessment of pelagic sharks caught in Atlantic pelagic longline fisheries. Aqua. Liv. Res. 22 (in press).

Dallas, J. F. 1992. Estimation of microsatellite mutation-rates in recombinant inbred strains of mouse. Mamm. Genome. 3:452-456.

Duncan, K. M., A. P. Martin, B. W. Bowen, H. G. De Couet. 2006. Global phylogeography of the scalloped hammerhead shark (*Sphyrna lewini*). Mol. Ecol. 15:2239-2251.

Edwards, S. and S. Bensch. 2009. Looking forwards or looking backwards in avian phylogeography? A comment on Zink and Barrowclough 2008. Mol. Ecol. 18:2930-2933.

Ellengren, H. 1995. Mutation-rates in procine microsatellite loci. Mamm. Genome. 6:376-377.

Endicott, P., S. Y. W. Ho, M. Metspalu, and C. Stringer. 2009. Evaluating the mitochondrial timescale of human evolution. TREE 24:515-521.

Excoffier, L., G. Laval, and S. Schneider. 2005. Arlequin ver. 3.0: An integrated software package for population genetics data analysis. Evol. Bioinformatics Online 1:47-50.

Fredsted, T., C. Pertoldi, M. H. Schierup, and P. M. Kappeler. 2005. Microsatellite analyses reveal fine-scale genetic structure in grey mouse lemurs (*Microcebus murinus*). Mol. Ecol. 14:2363-2372.

Fu, Y. X. 1996. New statistical tests of neutrality for DNA samples from a population. Genetics. 143:557-570.

Fu, Y. X., and W. H. Li. 1993. Statistical tests of neutrality of mutations. Genetics 133:693-709.

Gaggiotti, O. E., O. Lange, K. Rassmann, and C. Gliddons. 1999. A comparison of two indirect methods for estimating average levels of gene flow using microsatellite data. Mol. Ecol. 8:1513-1520.

Garza, J. C., and E. G. Williamson. 2001. Detection of reduction in population size using data from microsatellite data. Mol. Ecol. 10:305-318.

Gonser, R., P. Donnelly, G. Nicholson, and A. Di Rienzo. 2000. Microsatellite mutations and inferences about human demography. Genetics 154:1793-1807.

Henn, B. M., C. R. Gignoux, M. W. Feldman, and J. L. Mountain. 2009. Characterizing the time dependency of human mitochondrial DNA mutation rate estimates. Mol. Biol. Evol. 26:217-230.

Hasegawa, M., H. Kishino, and T. Yano. 1985. dating the human-ape split by a molecular clock of mitochondrial DNA. J. Mol. Evol. 22:160-174.

Hey, J. 2005. On the number of New World founders: a population genetic portrait of the peopling of the Americas. PLoS Biol. 3:e193.

Hey, J., and R. Nielsen. 2007. Integration with the Felsenstein equation for improved Morkov chain Monte Carlo methods in population genetics. PNAS 104:2785-2790.

Ho, S. Y., M. J. Phillips, A. Cooper, and A. J. Drummond. 2005. Time dependency of molecular clock rate estimates and systematic overestimation of recent divergence times. Mol. Biol. Evol. 22:1561-1568.

Howell, N., C. B. Smejkal, D. A. Mackey, P. F. Chinnery, D. M. Turnbull, and C. Herrnstadt. 2003. The pedigree rate of sequence divergence in the human mitochondrial genome. There is a difference between phylogenetic and pedigree rates. Am. J. Hum. Genet. 72:659-670.

IUCN, Species Survival Commission. 2007. Assessment of global status of pelagic and semi-pelagic sharks and rays. Available at www.iucn.org/media/ materials/ releases/?103/More-oceanic-sharks-added-to-the-IUCN-Red-List. Released February 2007.

Jackson, J. B. C., M. X. Kirby, W. H. Berger, K. A. Bjorndal, L. W. Botsford, B. J. Bourque, R. H. Bradbury, R. Cooke, J. Erlandson, J. A. Estes, T. P. Hughes, S. Kidwell, C. B. Lange, H. S. Lenihan, J. M. Pandolfi, C. H. Peterson, R. S. Steneck, M. J. Tegner, and R. R. Warner. 2001. Historical overfishing and the recent collapse of coastal ecosystems. Science 293:629-638.

Keeny, D. B., and E. J. Heist. 2003. Characterization of microsatellite loci isolated from the blacktip shark and their utility in requiem and hammerhead sharks. Mol. Ecol. Notes 3:501-504.

Klimley, A. P. 1981. Grouping behavior in the scalloped hammerhead. Oceanus 24: 65 71.

Klimley, A. P. 1985. Schooling in Sphyrna lewini, a species with low risk of predation: a non-egalitarian state. Zeitsch. Tierpsych. 70:297-319.

Klimley, A. P. 1987. The determinants of sexual segregation in the scalloped hammerhead shark, *Sphyrna lewini*. Enviro. Biol. .Fish. 18:27-40.

Klimley, A. P., and D. R. Nelson. 1981. Schooling of the scalloped hammerhead shark, *Sphyrna lewini*, in the Gulf of California. Fish. Bull. 79:356-360.

Klimley, A. P., and S. B. Butler. 1988. Immigration and emigration of a pelagic fish assemblage to seamounts in the Gulf of California related to water mass movements using satellite imagery. Mar. Ecol. Prog. Ser. 49:11-20.

Klimley, A. P., I. Cabrera-Mancilla, and J. L. Castillo-Geniz. 1993. Descripcion de los movimientos horizontales y verticales del tiburon martillo *Sphyrna lewini*, del sur de Golf de California, Mexico. Cien. Mar. 19:95-115.

Kohler, N. E., J. G. Casey, and P. A. Turner. 1998. NMFS cooperative shark tagging program, 1962-93: An atlas of shark tag and recapture data. Mar. Fish. Rev. 60:187.

Kordos, L. M., and R. S. Burton. 1993. Genetic differentiation of Texas Gulf-coast populations of the blue-crab *Callinectes sapidus*. Mar. Biol. 117:227-233.

Lessios, H. A. 2008. The great American schism: divergence of marine organisms after the rise of the Central American Isthmus. Ann. Rev. Ecol. Evol. System. 39:63-91.

Marko, P. B. 2002. Fossil calibration of molecular clocks and the divergence times of geminate species pairs separated by the Isthmus of Panama. Mol. Biol. Evol. 19:2005-2021.

Marko, P. B. and K. R. Barr. 2007. Basin-scale patterns of mtDNA differentiation and gene flow in the bay scallop *Argopecten irradians concentricus*. Mar. Ecol. Prog. Ser. 349:139-150.

Martin, A. P., G. J. P. Naylor, S. R. Palumbi. 1992. Rates of mitochondrial evolution in sharks are slow compared with mammals. Nature 357:153-155.

Martin, A. P. 1999. Substitution rates of organelle and nuclear genes in sharks: implicating metabolic rate (again). Mol. Biol. Evol. 16:996-1002.

Martínez-Ortíz, J., F. Galván-Magaña, M. Carrera-Fernández, D. Mendoza-Intriago, C. Estupiñán-Montaño, and L. Cedeño-Figueroa. 2007. Abundancia estacional de tiburones desembarcados en Manta – Ecuador. pp. 9-27 In: Tiburones en el Ecuador: Casos de Estudio. (Eds. J. Martínez-Ortíz, F. Galván-Magaña) EPESPO-PMRC. Manta, Ecuador.

Morreale, S. J., E. A. Standora, J. R. Spotila, and F. V. Paladino. 1996. Migration corridor for sea turtles. Nature 384:319-320.

Musick, J. A., G. Burgess, G. Cailliet, M. Camhi, and S. Fordham. 2000. Management of sharks and their relatives (Elasmobranchii). Fisheries 25:9-13.

Nance, H. A., T. S. Daly-Engel, and P. B. Marko. 2009. New microsatellite loci for the endangered scalloped hammerhead shark, *Sphyrna lewini*. Mol. Ecol. Res. 9:955-957.

Nance, H. A., and B. Sloop. *in prep*. Genetic estimates of current and historic effective population size in the endangered scalloped hammerhead shark, *Sphyrna lewini*. Mol. Ecol.

Niemiller, M. L., B. M. Fitzpatrick, and B. T. Miller. 2008. Recent divergence with gene flow in Tennessee cave salamanders (Plethodontidae: *Gyrinophilus*) inferred from gene genealogies. Mol. Ecol. 17:2258-2275.

Palumbi, S. R. 2003. Population genetics, demographic connectivity, and the design of marine reserves. Ecol. App. 13:S146-S158.

Palimbi, S. R., and R. R. Warner. 2003. Why gobies are like hobbits. Science 299:51-52.

Peakall, R., and P. E. Smouse. 2005. GenAlEx 6: genetic analysis in excel – Population genetic software for teaching and research. Australian National University, Canberra.

Pelc, R. A., R. R. Warner, and S. D. Gaines. 2009. Geographical patterns of genetic structure in marine species with contrasting life histories. J. Biogeogr. 36:1881-1890.

Perrin, C., S. R. Wing, and M. S. Roy. 2004. Effects of hydrogeographic barriers on population genetic structure of the sea star *Coscinasterias muricata* (Echinodermata, Asteroidea) in the New Zealand fiords. Mol. Ecol. 13:2183-2195.

Piercy, A. N., J. K. Carlson, J. A. Sulikowski, and G. H. Burgess. 2007. Age and growth of the scalloped hammerhead shark, *Sphyrna lewini*, in the north-west Atlantic Ocean and Gulf of Mexico. Mar. Fresh. Res. 58:34-40.

Pinnegar, J. K. and G. H. Engelhard. 2008. The 'shifting baseline' phenomenon: a global perspective. Rev. Fish. Biol. Fisheries 18:1-16.

Posada, D., and K. A. Crandall. 1998. Modeltest: testing the model of DNA substitution. Bioinfo. 14:817-818.

Pritchard, J. K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. Genetics 155:945-959.

Quattro, J. M., D. S. Stoner, W. B. Driggers, C. A. Anderson, K. A. Priede, E. C. Hoppmann, N. H. Campbell, K. M. Duncan, and J. M. Grady. 2006. Genetic evidence of cryptic speciation within hammerhead sharks (genus *Sphyrna*). Mar. Biol. 148:1143-1155.

Queller, D. C., and K. F. Goodnight. 1989. Estimating relatedness using genetic markers. Evolution 43: 258-275.

Rambaut, A., and A. J. Drummond. 2007. Tracer v1.4, Available from http://beast.bio.ed.ac.uk/Tracer.

Ramos-Onsins, S. E., and J. Rozas. 2002. Statistical properties of new neutrality tests against population growth. Mol. Biol. Evol. 19:2092-2100.

Rice, W. R. 1989. Analyzing tables of statistical tests. Evolution 43:223-225.

Rogers, A. R., and H. Harpending. 1992. Population growth makes waves in the distribution of pairwise genetic differences. Mol. Biol. Evol. 9:552-569.

Rooker, J. R., J. R. Alvarado Bremer, B. A. Block, H. Dewar, G. DeMetrio, A. Corriero, R. T. Kraus, E. D. Prince, E. Rodríguez-Marín, and D. H. Secor. 2007. Reviews in Fisheries Science 15: 265-310.

Rozas, J. J., C. Sánchez-DelBarrio, X. Messeguer, and R. Rozas. 2003. DnaSP, DNA polymorphism analices by the coalescent and other methods. Bioinfo. 19: 2496-2497.

Sotka, E. E., J. A. Hempelmann, and C. H. Biermann. 2005. Genetic evidence of postglacial population expansion in Puget Sound rockfish (*Sebastes emphaeus*). Mar. Biotech. 7:223-230.

Stevens, J. D. and J. M. Lyle. 1989. Biology of three hammerhead shatks (*Eusphyra blochii*, *Sphyrna mokarran*, and *S. lewini*) from Nothern Australia. Aust. J. Freshwater Res. 40:129-146.

Strasburg, J. L., and L. H. Rieseberg. 2009. How robust are "Isolation with Migration" analyses to violations of the IM model? A simulation study. Mol. Biol. Evol. (in press).

Swofford, D. L. 2002. PAUP*. Phylogenetic Analysis Using Parsimony (*and other methods). Version 4. Sinauer Associates, Sunderland, Mass.

Tajima, F., and M. Nei. 1984. Estimation of evolutionary distance between nucleotide sequences. Mol. Biol. Evol. 1:269-285.

Templeton, A. R., K. A. Crandall, and C. F. Sing. 1992. A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III Cladogram estimation. Genetics 132:619-633.

Thompson, J. D., T. J. Gibson, F. Plewniak, F. Jeanmougin, and D.G. Higgins. 1998. Multiple sequence alignment with Clustal X. Trends Biochem. Sci. 23:403-405.

Todd, C. D., W. J. Lambert, and J. P. Thorpe. 1998. The genetic structure of intertidal populations of two species of nudibranch mollusks with planktotrophic and pelagic lecithotrophic larval stages: are pelagic larvae 'for' dispersal? J. Exp. Mar. Biol. Ecol. 228:1-28.

Torres-Huerta, A. M. 1999. Observaciones sobre la biologia reproductiva de la cornuda barrosa, *Sphyrna lewini* (Griffith y Smith, 1834) (Pisces: Sphyrnidae) en aguas del noroeste de Mexico. Bachelor Sciences. UNAM. Escuela Nacional de Estudios Profesionales, Iztacala. 68 pp.

Van Oosterhout, C., W. F. Hutchinson, D. P. M. Wills, and P. Shipley. 2004. Micro Checker: software for identifying and correcting genotyping errors in microsatellite data. Mol. Ecol. Notes 4:535-538.

Vucetich, J. A., and T. A. Waite. 2000. Is one migrant per generation sufficient for the genetic management of fluctuating populations? Animal Conserv. 3:261-266.

Waits, L., P. Taberlet, J. E. Swenson, F. Sandegren, and R. Franzén. 2000. Nuclear DNA microsatellite analysis of genetic diversity and gene flow in the Scandinavian brown bear (*Ursus arctos*). Mol. Ecol. 9:421-431.

Weber, J. L., and C. Wong. 1993. Mutation of short human tandem repeats. Hum. Mol. Genet. 2:1123-1128.

Yue, G. H., P. Beeckmann, and H. Geldermann. 2002. Mutation rate at swine microsatellite loci. Genetica 114:113-119.

Zanella, I. 2008. Caracterización de la pesca y algunos aspectos sobre la reproducción, alimentación y distribución del tiburón martillo *Sphyrna lewini*, Sphyrnidae, en el Pacífico de Costa Rica. Tesis de M. en C., Universidad Nacional de Costa Rica, Instituto Internacional de Conservacion y Manejo de Vida Silvestre, 104 pp.

Zink, R. M., and G. F. Barrowclough. 2008. Mitochondrial DNA under siege in avian phylogeography. Mol. Ecol. 17:2107-2121.

Table 2.1: Location (longitude and latitude), number per year collected, and haplotype diversity and neutrality statistics of *Sphyrna lewini* samples in the Eastern Pacific. Site abbreviations correspond to locations in Figure 2.1. Nucleotide (π) and haplotype (h) diversities are shown. Neutrality statistics Fu's F_s and Fu and Li's D^*; none were significant at $\alpha = 05$. Though none are significant, only samples TAR and SCA show an increase in new mutations with negative D^* values.

Sample	Coordinates	Year(s)	n, msat	n, mtDNA[1]	π	h	F_s	D^*
LAP	N 24.20, W 110.40	2006/07	30/24	17	0.000807	0.4412	5.435	0.677
MAZ	N 23.20, W 106.40	2006/07	14/38	22	0.00076	0.4156	5.709	0.064
TAR	N 9.80, W 84.80	2007/08	40/40	20	0.00132	0.6158	4.087	-1.193
SCA	N 7.56, W 81.30	2007/08	46/46	22	0.001331	0.5974	2.869	-1.729
CEB	N 7.55, W 81.00	2008	21	18	0.00116	0.5686	4.196	-0.552
GPA	N 7.01, W 78.19	2008	9	7	0.001045	0.5714	4.276	0.953
MAN	S 1.10, 84.95	2007/08	36/43	20	0.000962	0.5263	6.643	0.650

[1]All mtDNA samples from 2007 except CEB and GPA

Table 2.2: AMOVA results for samples collected at sites in different years, characterizing temporal differentiation with microsatellites. Only sites where collecting occurred in two years are shown (i.e. Cebaco Island (CEB) and Gulf of Panama (GPA) omitted).

Samples	Source of variation	d.f.	SS	Variance components	% Var.	F_{ST}
LAP 2006/07	among pops	1	7.296	Va 0.040	0.77	0.008*
	within	106	547.362	Vb 5.164	99.23	
MAZ 2006/07	among pops	1	7.390	0.046	0.83	0.009*
	within	103	561.244	5.502	99.17	
TAR 2007/08	among pops	1	7.662	0.026	0.46	0.005*
	within	158	886.450	5.610	99.54	
SCA 2007/08	among pops	1	4.174	-0.011	-0.21	0.000
	within	182	937.272	5.150	100.21	
MAN 2007/08	among pops	1	6.599	0.019	0.36	0.054
	within	186	801.641	5.139	99.64	

* indicates significant at $\alpha = 0.05$.

Table 2.3. AMOVA results for all sites, characterizing spatial structure with both mtDNA (Φ_{ST}) and microsatellites (F_{ST}). Sample site abbreviations are followed by 07 and/or 08 indicating which years (2007 and/or 2008) are included in the samples analyzed.

Samples included	Marker	Source of variation	d.f.	SS	Variance components	% Var.	Φ_{ST}/F_{ST}
LAP07, MAZ07, TAR07	mtDNA	among pops	6	2.746	Va 0.009	3.13	0.031
SAC07, CEB08, GPA08, MAN07		within	119	34.57	Vb 0.291	96.87	
LAP07, MAZ07, TAR07	msats	among pops	6	43.293	Va 0.024	0.46	0.005*
SAC0708, CEB08, GPA08, MAN0708		within	599	3147.33	Vb 5.254	99.54	

* indicates significant at α = 0.05.

Table 2.4: Pairwise locus-by-locus AMOVA results characterizing structure based on microsatellites (F_{ST}) between all Eastern Pacific sites. Because temporal structure was detected between years from sites LAP, MAZ, and TAR, separate pairwise tests were run with samples from each year from these sites (2006 and 2007 for LAP and MAZ, 2007 and 2008 for TAR). Different years, and their associated pairwise F_{ST} values, are distinguished by italics. Values significant at $\alpha = 0.05$ are indicated in bold.

	LAP2007 ***LAP2006***	**MAZ2007** ***MAZ2006***	**TAR2007** ***TAR2008***	**SCA**	**CEB**	**PAN**	**MAN**
LAP2007 ***LAP2006***	*						
MAZ2007 ***MAZ2006***	0.000 ***0.011***	*					
TAR2007 ***TAR2008***	**0.01** **(LAP07)*0.006***	**0.007** **(MAZ07)*0.005***	*				
SCA	0.005 ***0.013***	**0.005** ***0.010***	**0.007** ***0.005***	*			
CEB	**0.015** ***0.016***	**0.012** ***0.018***	**0.013** ***0.010***	**0.011**	*		
PAN	0.012 *0.009*	**0.014** ***0.016***	0.009 *0.001*	0.005	0.000	*	
MAN	**0.006** ***0.018***	**0.004** ***0.013***	**0.009** ***0.007***	**0.002**	**0.007**	0.009	*

Table 2.5. Tau (τ) and 90% confidence intervals of simulations under the model of sudden expansion implemented in the mismatch distribution analysis in Arlequin. Harpending's raggedness index (*r*), time since population expansion (*t*), and associated 90% confidence intervals are shown for all populations where the sudden expansion hypothesis could not be rejected. Time since expansion (*t*) was estimated using the slow, fossil-calibrated mutation rate and the faster, Isthmus-calibrated rate.

Sample	τ	90% CI	*r*	*t* (slow μ)	90% CI	*t* (fast μ)	90% CI
LAP	0.641	0.042 - 1.277	0.208	182,102	12,428 - 369,075	146,347	9,811 - 291,553
MAZ	0.598	0.105 - 1.191	0.201	169,886	30,347 - 344,220	136,530	23,973 - 271,918
TAR	0.898	0.336 - 1.617	0.140	255,114	97,110 - 467,341	205,023	76,712 - 369,178
SCA	0.867	0.375 - 1.578	0.149	246,307	108,382 - 456,069	197,945	85,616 - 360,274
CEB	0.812	0.281 - 1.559	0.201	230,682	81,214 - 450,578	185,388	64,155 - 355,936
GPA	0.898	0 - 22.75	0.347	255,114	0 - 6,575,144	205,023	0 - 5,194,064
MAN	0.814*	0.313 - 1.414	0.280	NA	NA	NA	NA

* indicates significant at α = 0.05.

Table 2.6. Results from MSVAR (Beaumont, 1999) analyses using only microsatellite data show current (N_{e0}) and historic (N_{e1}) estimates of effective population size, and time in years (t) since the onset of population decline. All point estimates are followed by 95% highest posterior density intervals, as calculated in Tracer v. 1.4.1 (Rambaut and Drummond, 2008).

	N_{e0}	N_{e1}	t (in years)
Population	**95% HPD**	**95% HPD**	**95% HPD**
LAP	435.51	39627.80	8452.79
	36.16 - 4717.37	4718.46 - 324041.03	493.06 - 117733.49
MAZ	384.68	43551.19	6181.59
	28.89 - 4627.01	4927.20 - 365426.47	386.99 - 81320.49
TAR	481.95	34994.52	5766.34
	49.57 - 4607.87	4102.99 - 289867.82	347.46 - 86616.37
SCA	1003.46	54175.13	15808.84
	123.68 - 8150.80	5931.98 - 466874.33	661.46 - 220140.53
CEB	226.67	38256.04	3639.15
	8.00 - 4952.22	4463.75 - 333042.76	116.33 - 79031.46
MAN	1015.08	35318.32	16722.45
	121.03 - 8483.99	4131.43 - 301647.69	987.87 - 229034.02

Table 2.7: $\Theta = 4N_e\mu$ for populations 1, 2, and the ancestral population from which they arose, migration parameters m_1 and m_2, and time in years (t) since populations diverged using both the slower fossil-calibrated mutation rate (3.21×10^{-9} subs/year) and the faster Panamanian Isthmus-calibrated mutation rate (4.00×10^{-9} subs/year). 90% HPD represents the interval on the x-axis where 90% of the area under the posterior probability density curve lies. Upper boundaries of ∞ indicate the HPD had not yet reached zero, though was approaching it (in all cases except Θ_{2CEB} in the SCA-CEB comparison). In each pair of populations, population 1 is listed first.

Samples	Θ_1	Θ_2	Θ_A	m_1	m_2	t/μ	"Slow" rate t, years	"Fast" rat t, years
LAP-MAZ	0.04	0.40	87.50	184.40	35.55	0.01	97.60	77.10
90% HPD	0.01 - 4.00	0.08 - 8.00*	51.70 - 129.70	96.4 - 739.6*	13.05 - 778.95*	0.00 - 0.07	46.47 - 1,084.39	36.71 - 856.
MAZ-TAR	0.05	1.15	89.55	57.05	14.63	0.01	547.75	432.69
	0.02 - 1.76	0.09 - 11.99*	56.85 - 150.45	16.45 - 353.85	0.23 - 449.78	0.00 - 0.23	190.52 - 10,716.76	150.50 - 8,46
TAR-SCA	0.61	1.26	85.41	30.38	4.42	0.03	38,089.51	30,088.97
	0.13 - 2.13	0.33 - 3.01	47.90 - 132.84	6.83 - 110.33*	0.05 - 41.09	0.01 - 0.27	15,350.98 - 366,193.87	12,669.04 - 289,
SCA-CEB	0.05	19.99	67.74	54.80	48.65	0.01	781.50	617.35
	0.02 - 0.72	4.01 - 19.99*	48.42 - 129.38	6.8 - 565.2*	12.95 - 536.55*	0.00 - 0.16	293.06 - 15,630.06	231.51 - 12,34
CEB-MAN	0.20	0.53	83.07	66.75	19.21	0.06	1,636.21	1,292.53
	0.05 - 13.99*	0.11 - 8.30	58.11 - 122.07	14.75 - 241.75	0.115 - 166.64	0.01 - 0.16	289.60 - 4,633.53	228.77 - 3,66

Figure 2.1. Map of Eastern Pacific range of *Sphyrna lewini* and study area. Sample localities and their associated abbreviations indicated by black dots. The three Panamanian sites are enlarged due to their close proximity to one another.

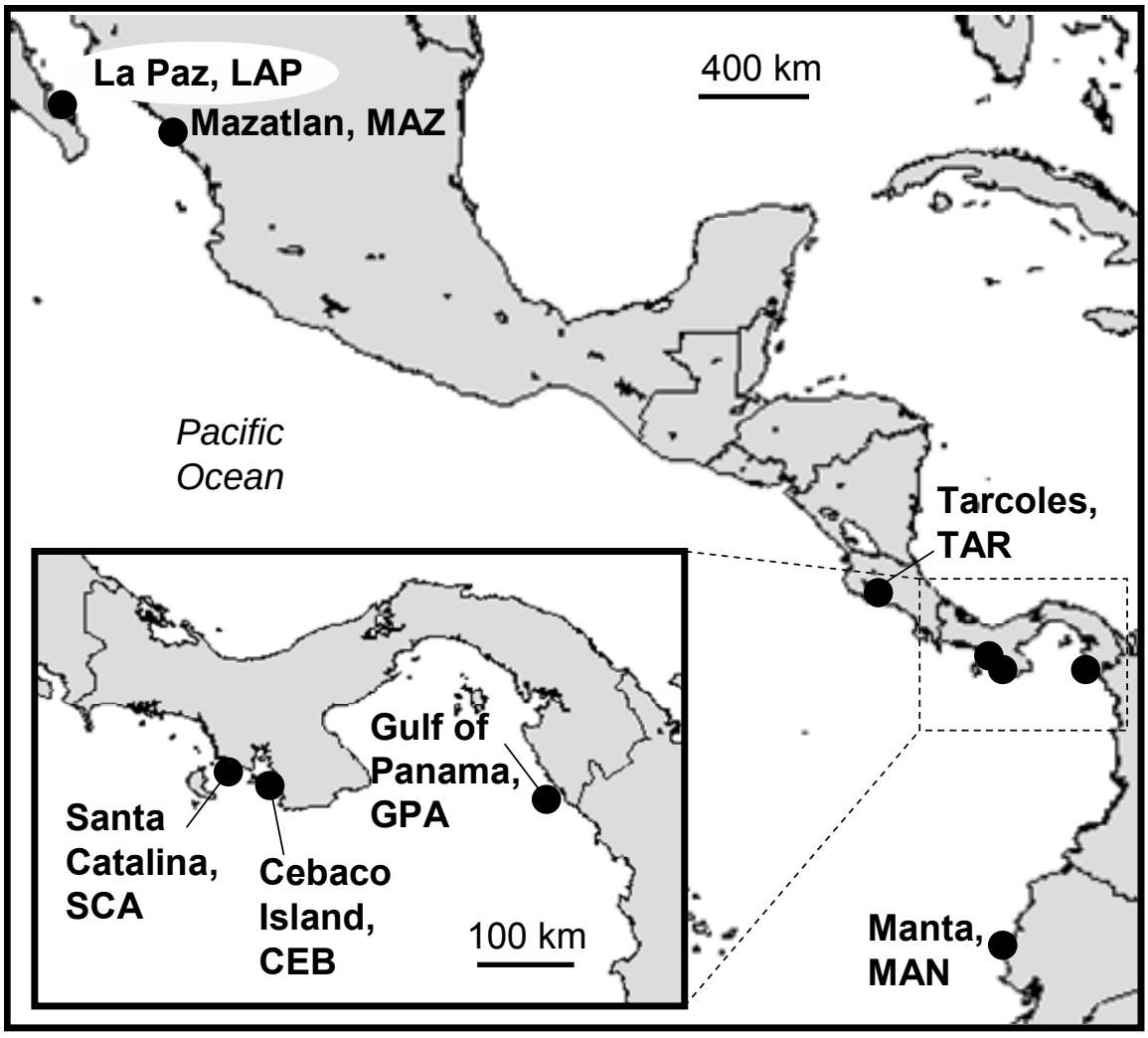


Figure 2.2. Haplotype network showing proportion of haplotypes per population. Haplotypes A and B are common to all populations. Haplotype C is shared by TAR and SCA, haplotypes D and E are unique to TAR and CEB, respectively, and haplotypes F and G are unique to SCA. Numbers inside haplotypes C through G indicate the number of haplotypes present in our sampled individuals.

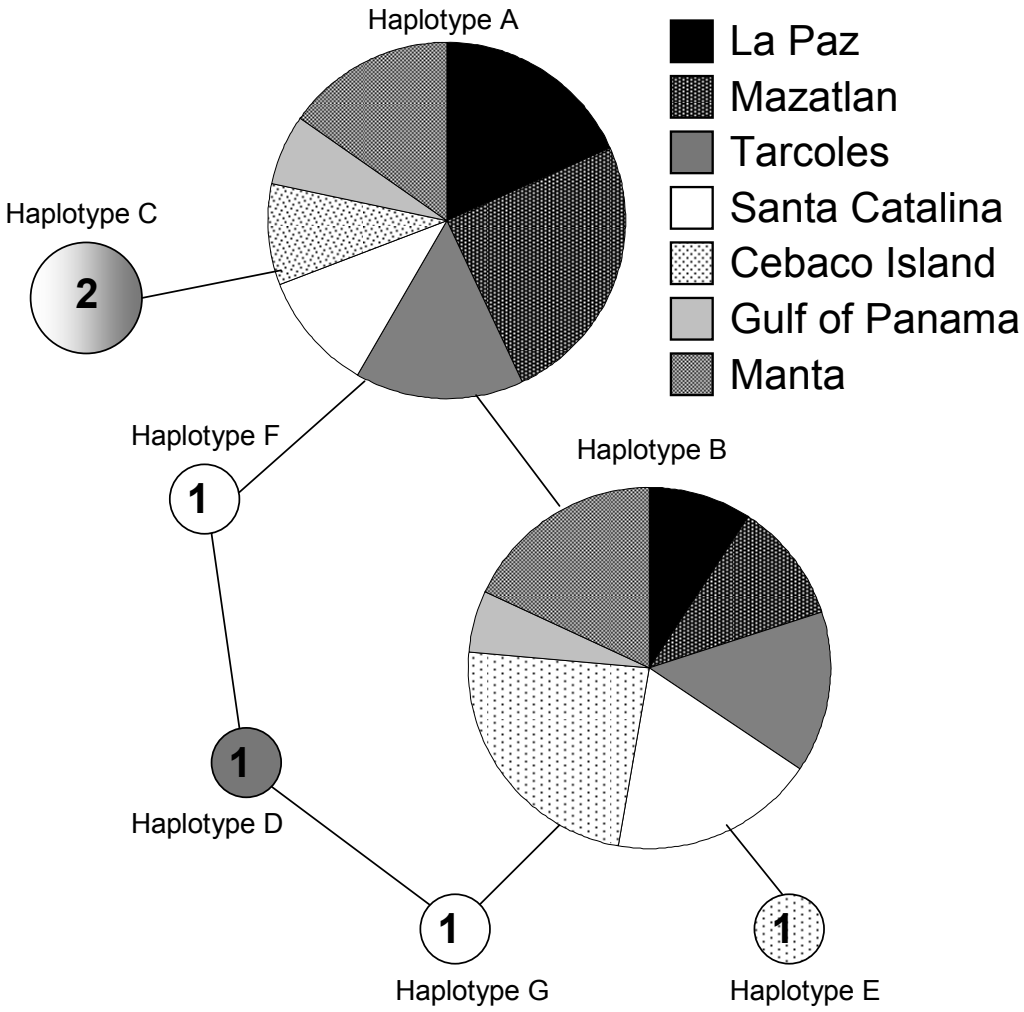


Figure 2.3. Observed (black) and expected (gray) distribution of pairwise nucleotide differences between haplotypes (mismatch distributions) for each population under the model of demographic expansion. All but Manta, Ecuador are consistent with the population expansion model (MAN p = 0.044).

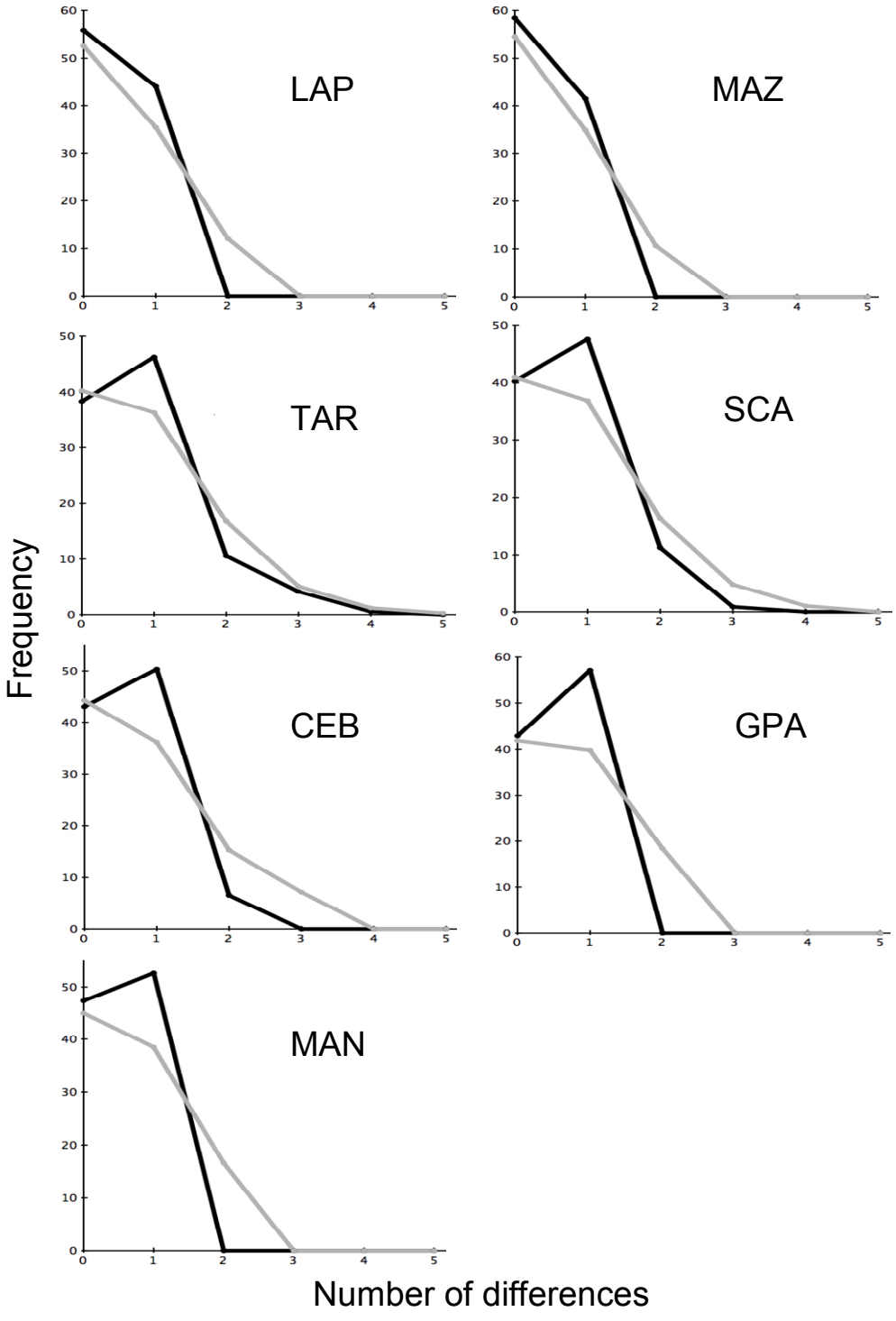


Figure 2.4. M ratio test results for each population, showing the population-specific M ratio (open circles), average M from simulations assuming each population is in drift-mutation equilibrium (black circles), and critical M_c based on these simulations (gray circles). M values below M_c indicate a population has undergone a recent bottleneck. All data shown here were calculated with a proportion of single step mutations (p_s) of 0.90 and an average size of mutations evolving more than one repeat unit (Δ_g) of 3.5. All M values were calculated with θ = 0.01, 0.1, 1.0, and 10.0, corresponding to N_e = 1445, 14,451, 144,509, and 1,445,087, respectively.

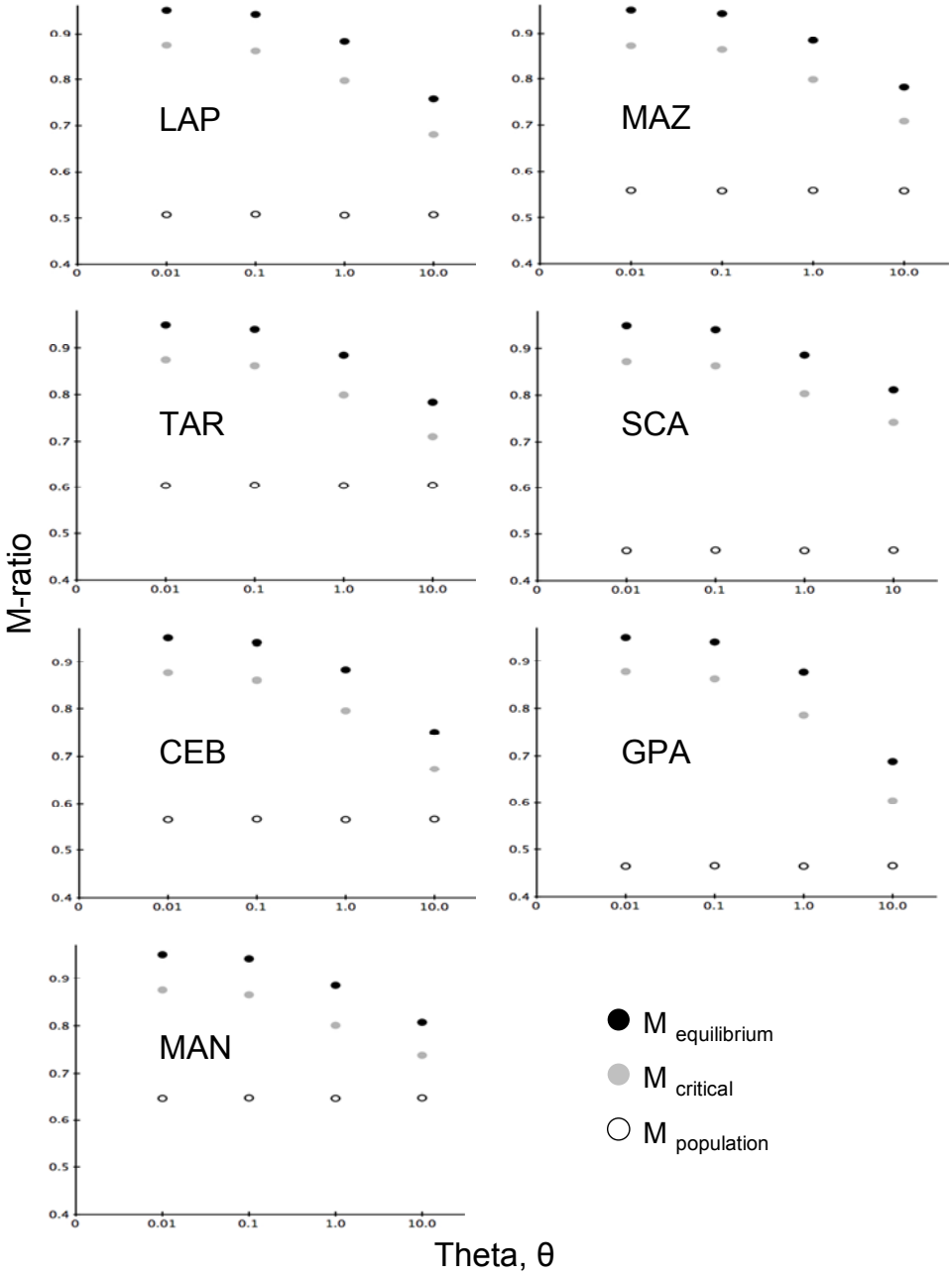


Figure 2.5. Posterior probability density of time since divergence for each population pair analyzed in IMa. Time (t) is in years. Black represents the posterior probability density (PPD) of time based on the slower fossil-calibrated mutation rate (3.21×10^{-9} subs/year) and gray represents the PPD based on the faster Panamanian Isthmus-calibrated rate (4.00×10^{-9} subs/year).

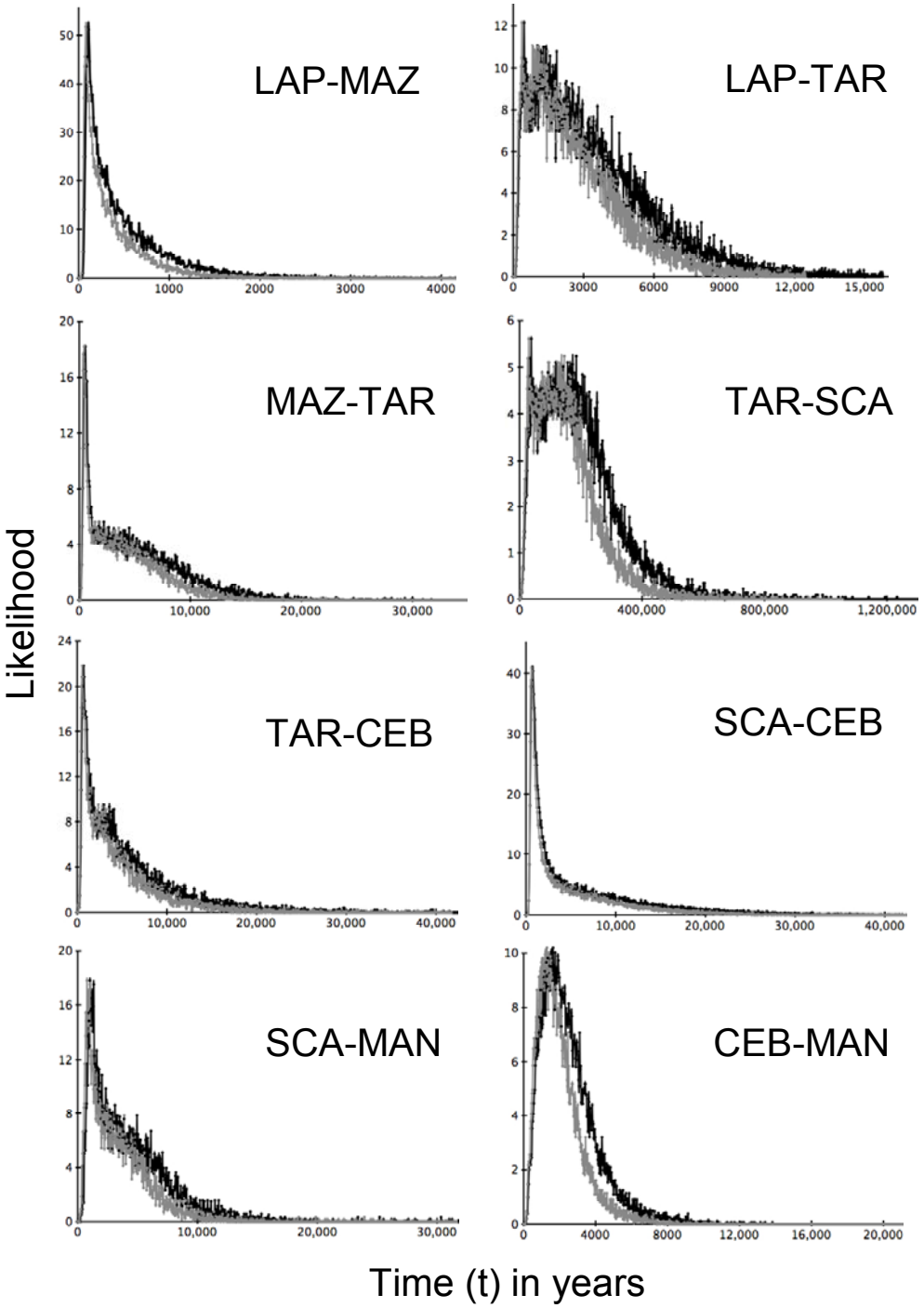
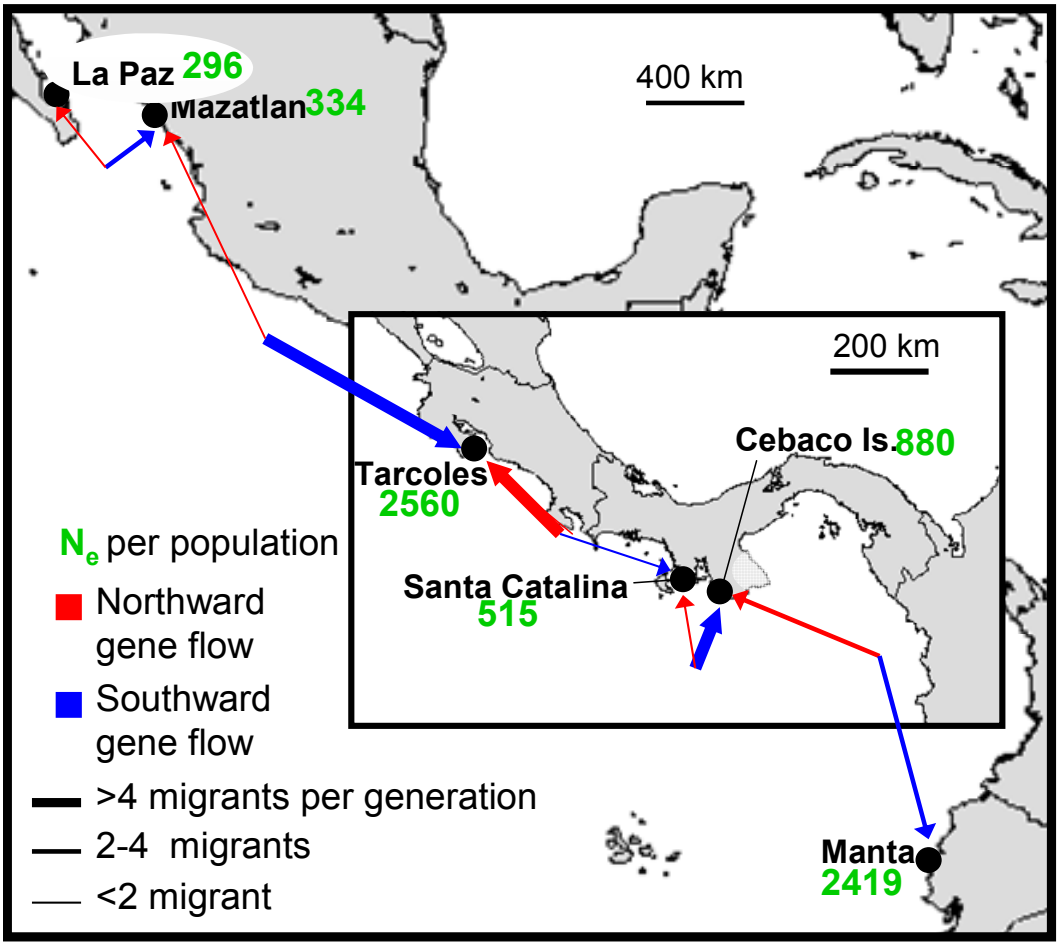


Figure 2.6. Map showing relative migration rates (Nm = the number of migrants per generation) between adjacent pairs of EP populations. Red arrows indicate northward gene flow; blue indicate southward flow. Thickness of arrows corresponds to magnitude of flow, or number of migrants per generation. Values in green indicate current N_e, as averaged from estimates of MSVAR and IMa. N_e from IMa was calculated with the equation $\Theta = 4N_e\mu$, where both the fossil- and Isthmus-calibrated μ were used for two estimates of N_e.



CHAPTER THREE

ESTIMATING CURRENT AND HISTORIC EFFECTIVE POPUALTION SIZE OF THE ENDANGERED SCALLOPED HAMMERHEAD SHARK, *SPHYRNA LEWINI*

Abstract

Obtaining precise and unbiased estimates of N_e is challenging; we therefore used four contemporary genetic methods (Linkage Disequilibrium, Sibship Assignment, Heterozygote Excess, and Temporal), and two coalescent approaches implemented in the programs MSVAR and IMa to estimate N_e for the endangered scalloped hammerhead shark, *Sphyrna lewini*, throughout its Eastern Pacific (EP) range. Because *S. lewini* has overlapping generations, single-sample contemporary estimates represent N_b, the effective number of breeders, yet considering the relationship between N_b and N_e ($N_b \leq N_e \leq N_b$ x G, where G is generation time in years), 95% confidence intervals of contemporary estimates overlapped with the 95% posterior probability densities of coalescent-based estimates of N_e. N_b varied, at times considerably, between cohorts from the same location. Although this could be caused by different parental populations producing each cohort, or by fluctuations in population size, larger sample sizes and greater precision in our contemporary estimates are necessary to make such biological inferences with greater confidence. Estimates of current and ancestral N_e indicate EP *S. lewini* experienced severe population decline in the past several centuries. Yet the most interesting result is the agreement between contemporary and coalescent estimates of current N_e, suggesting that population decline was sudden and the post-reduction N_e has remained fairly constant since.

Introduction

Effective population size (N_e) is arguably one of the most important demographic parameters for species of conservation concern. Yet, obtaining accurate and precise estimates of N_e in nature is extremely difficult. This difficulty is caused not by a lack of methods to generate estimates of N_e, but arises rather because the data required for such analyses are challenging to collect. Though much recent attention has focused on various genetic methods to estimate N_e [see reviews by Beaumont (2003) and Wang (2005)], results can be unreliable and even misleading if assumptions pertinent to each method are not met. However, these methods are often the only option for estimating this parameter, particularly in small or declining populations of conservation concern.

For threatened or endangered marine fishes capable of swimming vast distances in deep off-shore waters, genetic methods are far easier to implement than more traditional capture-recapture methods for estimating N. As such, they are becoming increasingly common in studies of commercially important species [e.g. Pacific salmon: Waples (1990, 2002); brown trout: Hansen et al. (2000); red drum: Turner et al. (2002); darkblotched rockfish: Gomez-Uchida and Banks (2006)]. For the globally endangered (IUCN 2007) scalloped hammerhead shark, *Sphyrna lewini*, reliable estimates of N_e are paramount, as no current estimates of population sizes are known. The fins of this shark, together with *Sphyrna zygaena*, comprise roughly 49-90 thousand tons in the Chinese fin trade, from an estimated 1.3 to 2.7 million sharks annually (Clarke et al. 2006). Like most chondrichtheys, *S. lewini* reaches sexual maturity at a late age, is less fecund than most bony fish, and therefore less able to recover from over-exploitation (Musick 2000).

Despite its IUCN Red List status, no species-specific management plan exists anywhere for this cosmopolitan, tropical shark (Compagno 1984) caught both intentionally and as by-catch throughout its range (Dulvy et al. 2008). Declines in *S. lewini* have been documented globally (e.g. Bonfil et al. 2005; Dudley and Simpfendorfer 2006; Zeeburg et al. 2006), yet with neither estimates of current nor historic effective population size, the magnitude of decline and urgency with which conservation plans must be implemented has hindered appropriate management action.

With life history and reproductive biology parameters taken into account, we have used six different genetic methods to estimate N_e for six populations of *S. lewini* throughout its Eastern Pacific (EP) range. Specifically, we applied four methods for estimating contemporary N_e, or N_e that applies to the time at sampling (Waples 2005), and two methods that rely on the coalescence of gene genealogies to estimate both current and historic N_e. Sample collection, genotyping, sequencing, and coalescent-based analyses were completed previously as part of a larger spatial genetic structure study across the EP range of *S. lewini* (Nance et al. *in prep.*). The current study added the contemporary estimates of N_e, and our intent was to use several different genetic methods to generate this population parameter for *S. lewini*, and in the process, determine whether estimates were congruent across different methods.

Methods

Reproductive Biology of *Sphyrna lewini*

Genetic methods for estimating contemporary N_e are sensitive to generation time and several aspects of an organism's reproductive biology. Therefore, we needed to consider several life history attributes of *S. lewini* in our analyses. *Sphyrna lewini* is viviparous with a yolk-sac placenta and litter sizes of 15 to 31 pups (Compagno 1984). While females tend to be found farther offshore than males (e.g. Klimley 1981, 1987; Branstetter 1987; Stevens and Lyle 1989; de Bruyn et al. 2005), pregnant females move into protected bays and coastal areas for parturition (e.g. Clarke 1971; Compagno 1984; Castro 1993) after a gestation period of ten to 12 months (Stevens and Lyle 1989; Liu and Chen 1999; Hazin et al. 2001). Evidence of simultaneous development of ovarian follicles and embryos, and the noted presence of spermatozoa in gravid females suggest annual reproduction (Hazin et al. 2001; de Bruyn et al. 2005; Castro 2009).

Litters are roughly comprised of equal numbers of males and females (Liu and Chen 1999; Torres-Huerta 1999; Bejarano-Álvarez 2007), however these equal sex ratios change as sharks mature. While juvenile *S. lewini* remain in protected coastal areas until reaching larger sizes, females move offshore earlier than males (e.g. Klimley 1987; Stevens and Lyle 1989). Sexual maturity occurs at roughly 200 - 250cm for females and 180 - 200cm for males (Branstetter 1987; Stevens and Lyle 1989; Hazin et al. 2001), though there is disagreement regarding the age at maturity. This disagreement stems from the method by which age is determined; that is counting the growth bands of vertebral centra. Age is determined by assuming one band per year (e.g. Branstetter 1987; Conrath et al. 2002; Sulikowski et al. 2005; Piercy et al. 2007) or two bands per year (e.g. Pratt and Casey 1983; Chen et al. 1990; Tolentino and Mendoza 2001;

Ainslado-Tolentino et al. 2008). For our analyses, we have adopted the one-band-per-year growth model, as this assumption is most widely applied in analyses of age and growth in elasmobranches, and a previous analysis on *Isurus oxyrinchus* assuming two-band-per-year growth (Pratt and Casey 1983) was recently revisited using bomb radiocarbon methods and it was found that growth-band deposition is actually annual for this species (Campana et al. 2002). With a one-year band growth model, age at maturity is roughly 15 years for females and nine to ten years for males (Branstetter 1987). Maximum age in both sexes is roughly 30 years (Piercy et al. 2007), thus generation time can range between 20 to 30 years.

Sample Collection, DNA Extraction, Genotyping, Sequencing

A total of 429 tissue samples used in this study were collected for a previous study (Nance et al. *in prep*) from artisanal fishers from six Eastern Pacific sites: La Paz and Mazatlan (Mexico), Tarcoles (Costa Rica), Santa Catalina and Cebaco Island (Panama), and Manta (Ecuador) between 2001 and 2008 (see Figure 1 and Table 1). DNA extraction, genotyping, and mtDNA sequencing methods are described in Nance et al. (*in prep*). Tests for null alleles, departures from Hardy-Weinberg equilibrium, and linkage disequilibrium for the 15 microsatellite loci used were implemented in the programs Micro-Checker v. 2.2.3 (Van Oosterhout et al. 2004) and Arlequin v. 3.11 (Excoffier et al. 2005). Tests of neutrality applied to the mtDNA sequence data included Tajima's D (Tajima 1989) and Fu's F_s (Fu 1996), calculated in Arlequin, each with 10,000 simulations. All tests are described in detail in Nance et al. (*in prep*).

Age Classes

Because contemporary estimates of N_e are sensitive to genetic change across successive generations, it was necessary to divide our sampled populations into discrete age classes. We first converted all measurements of total length (TL) to fork length (FL) using the linear regression equation TL = 1.296FL + 0.516 (Piercy et al. 2007). We then used estimates of sex-specific asymptotic size, growth coefficients, and size at year zero, calculated from the von Bertalanffy growth model by Piercy et al. (2007) to assign each individual to appropriate age classes based on their FL. All individuals collected from the same location in the same year that were in the same age class were grouped as a cohort for subsequent analyses for estimating contemporary N_e. In some cases, we had individuals from the same location collected in successive years that could be grouped in the same cohort (i.e. age class 2 individuals from 2008 were grouped with age class 1 individuals from 2007), to increase sample sizes.

Effective N_e Versus N_b

In iteroparous species with overlapping generations, all single-sample contemporary genetic methods of estimating N_e should actually be interpreted as N_b, the effective number of breeders that contributed to the sampled individuals (e.g. Waples 2005; Wang 2009). The relationship between the two can be summarized as $N_b \leq N_e \leq N_b \times G$, where G is the mean generation time (Wang 2009). In order to make estimates of N_e comparable across different methods, we calculated the interval for $\hat{N}_e$ based on this relationship and multiplied all estimates of N_b by G [calculated using Felsenstein's

equation (1971); described below]. Both the lower boundary of $\hat{N}_e$ (that is, $\hat{N}_b$) and the upper boundary of $\hat{N}_e$ (that is, $\hat{N}_b$ x G) were reported for each contemporary method used. However, we refer to the estimates of effective population size generated by each method as $\hat{N}_e$ rather than $\hat{N}_b$ for consistency.

Methods of Estimating Contemporary N_e

Linkage Disequilibrium (LD) Method. The amount of linkage disequilibrium (LD, the non-random association of alleles at different loci) between two neutral markers is determined by the rate of recombination between the loci and N_e (Hill 1981). With our genotype data from discrete cohorts for all populations, we used the software LDNe v. 1.31 (Waples and Do 2008) to calculate $\hat{r}^2$, the mean squared correlation of allele frequencies at different gene loci (Waples 2006), and to estimate N_e. LDNe relies on an empirically-derived adjustment to $\hat{r}^2$ that eliminates the downward bias in estimates of N_e that arises when sample size (s) is smaller than actual N_e (Waples 2006). LDNe also considers low-frequency alleles and how they will affect precision and bias in estimates of N_e. To account for these alleles in our data, we used the "rule of thumb" suggested by Waples and Do (*in press*) for selecting a cut-off value (P_{crit}) for the inclusion of low-frequency alleles: for $s > 25$, $P_{crit} = 0.02$, and for $s \leq 25$, P_{crit} chosen so that $1/(2s) < P_{crit} \leq 1/s$. We first used LDNe to calculate N_b for each cohort per site individually and then combined cohorts from consecutive years per site to see whether the number of breeders changed when cohorts were combined.

Sibship Assignment (SA) Method. We used the program COLONY2 (Wang 2009) to estimate N_e based on sibship reconstructions of our sampled cohorts. COLONY2 uses an algorithm that searches for the maximum likelihood configuration of sibship assignments for all individuals in a sample based on their genotypes. This program is unique in that it considers the frequency of errors (estimated by the user) due to allele dropout (Class I), mutation, PCR error, miscalling, and data entry (Class II) when assigning sibship (Wang 2004). Based on the sibship reconstructions, COLONY2 then estimates N_e with the premise that the more pairs of siblings there are in a population, the smaller N_e is because all juveniles came from a smaller number of (the same) adults.

All of our cohorts consisted of juveniles, so we had no paternal or maternal data to add to the COLONY2 analyses. However, whether siblings are maternal or paternal has no effect on $\hat{N}_e$ for a randomly-mating population and non-random mating has little effect on $\hat{N}_e$ based on simulations (Wang 2009). For each cohort analyzed, we had allele frequencies updated during the course of five long runs with high full-likelihood precision. We set the frequency of errors to 5%, set no prior on the size of sibling groups, and chose polygamous mating systems for both males and females. In simulations by Wang (2009) to test how violations of these assumptions affect $\hat{N}_e$, populations under polygamous, maximal sibling mating systems led to an overestimate of the probability of sibship, and a downward bias in $\hat{N}_e$. However, this bias was small (see Wang 2009), so violations of our assumptions of polygamy and random mating should not strongly affect $\hat{N}_e$.

Heterozygote Excess (HE) Method. The heterozygote excess method (Pudovkin et al. 1996; Luikart and Cornuet 1999), based on the observed excess in heterozygosity in offspring when the number of breeders is small (Robertson 1965), was also implemented with the program COLONY2 (Wang 2009). Using the same input files and parameters as for the SA method analyses, COLONY2 also estimates N_e using the HE method. As with the SA method, we estimated N_e for each cohort sampled from all populations in all years. However, unlike the SA method, the HE method is quite sensitive to departures from random mating as this can lead to heterozygote deficiency or excess (Wang 2009).

Temporal Method (TM). This method is based on the relationship between N_e and genetic drift, and the increased magnitude of allele frequency changes when N_e is small (Krimbas and Tsakas 1971; Nei and Tajima 1981). Two samples from the same population separated by at least one generation are required to measure temporal genetic drift and estimate N_e (e.g. Waples 1989), unlike the three previous methods which only require one sample. While we had our sampled populations divided into different age classes, *S. lewini* is a species with overlapping generations and the TM assumes discrete generations. To accommodate, we used the correction method of Jorde and Ryman (1995). This method implements the equation:

$$\hat{N}_e = \mathrm{C}/(2\mathrm{GF}'),$$

where C is the correction factor obtained using survival and birth rate data, G is the mean generation length (Felsenstein 1971), and F' is the overall measure of temporal allele frequency change across two samples. To obtain values for these variables, we first had to calculate age-specific survival and birth rates for *S. lewini*. While no such data exist

for Eastern Pacific *S. lewini*, we obtained survival rates for *S. lewini* in the East Atlantic (Cortes et al. 2009). For age specific reproductive rates, we use 15 years as the age of first reproduction, and a mean litter size of 23 pups, which remains relatively constant throughout adulthood (Nguyen and Piercy *unpub. data*). From these data, we were able to calculate l_i (age-specific survival rates), b_i (birth rates), and p_i (probability of a gene being inherited from a parent of age *i*), for all age classes, *i*, and then used these data to calculate the correction factor, C. For this calculation, we used Jorde and Ryman's (1995) Equation 23, $C = \frac{f_{1,1}(t) + f_{1,1}(t+1) - 2f_{1,2}(t+1)}{f_{1,1}(t+1) - f_{1,1}(t)}$ where $f_{i,j}(t)$ is an estimate of the genetic drift variance between age classes *i* and *j* for cohort *t*. With $f_{i,j}(t=0)$ initially set to zero, Jorde and Ryman's (1995) Equations 10-13,

$$f_{1,1}(t+1) = 1 + \sum_{i=1}^{k}\sum_{j=1}^{k} p_i p_j f_{i,j}(t)$$

$$f_{i,i}(t+1) = \frac{1}{l_i} - \frac{1}{l_{i-1}} + f_{i-1,i-1}(t) \text{ for } 1 < i \le k$$

$$f_{1,j}(t+1) = \sum_{i=1}^{k} p_i f_{i,j-1}(t), \text{for } 1 < j \le k$$

$$f_{i,j}(t+1) = f_{i-1,j-1}(t), \text{ for } 1 < i < j \le k,$$

were iterated through using MATLAB v. 2007a on a Windows XP operating system. Computational accuracy was ensured by first validating Jorde and Ryman's (1995) results. Jorde and Ryman (1996) report approximately 50 iterations are needed before a constant value of C is obtained, however our data for *S. lewini* required 100 iterations

before equilibrium was reached. We also used MATLAB to calculate mean generation time $G = \sum_{i=1}^{k} p_i i$ based on the equation of Felsenstein (1971).

To calculate F', the change in allele frequency over time for each population, we used the program TempoFs (Jorde and Ryman, 2007). This program estimates genetic drift (F_s') corrected for the appropriate sampling plan [either Plans I or II of Nei and Tajima (1981) and Waples (1989)]. While this program also estimates N_e, it assumes discrete generations, and therefore the estimate was not appropriate for our data. To generate estimates of drift (F_s') our input included genotypic data for successive cohorts in each population and an indication of sampling Plan II (individuals were sampled and not replaced in the population). The program jackknifed over all loci to estimate a mean F_s' and generated a 95% confidence interval around this mean. For each EP population analyzed, we used different cohorts from two consecutive years to estimate the amount of drift, and ultimately N_e. Though we had cohorts from La Paz, MX across four years, only samples from 2006 and 2007 were analyzed as they were the only consecutive samples.

Coalescent Based Estimators of N_e

Detailed descriptions of our methods to estimate long-term current and historic N_e based on coalescent models are provided in Nance et al. (*in prep*). Here we provide a brief description of the two programs used previously in that study.

MSVAR. This program (v. 1.3) relies on Markov Chain Monte Carlo (MCMC) simulations of mutation and coalescent events that resulted in the present day genotypes in a sampled population (Beaumont 1999). The method assumes microsatellites have evolved via the single-step mutation model (SSM). Random samples from the Bayesian posterior distributions of demographic and mutational parameters were drawn and their likelihoods were calculated (Beaumont 1999). With this program, we estimated the posterior distribution of the parameters N_{e0} (current population size), N_{e1} (ancestral population size), μ (mean mutation rate of all loci), and t (time since population growth or decline) for each population.

IMa. To estimate current and ancestral N_e using both microsatellite and mtDNA control region sequence data, we used the program IMa (Hey and Nielson 2004) operated by the CBSU cluster computing system at Cornell University. This coalescent-based program simulates gene genealogies using Markov Chain Monte Carlo (MCMC) methods. The "isolation with migration" model implemented in IMa does not assume drift, migration, and mutation are in equilibrium, making it more appropriate for recently diverged populations that share haplotypes and alleles due to both recent gene flow and ancestral polymorphism. IMa estimates the population parameters Θ_1, Θ_2, and Θ_A (corresponding to two current populations in a pairwise comparison, and the ancestral population from which they arose, respectively), *m* (migration), and *t* (time since divergence). For our analyses regarding N_e, we were primarily concerned with estimates of population-specific Θ given the relation $\Theta = 4N_e\mu$.

With estimates of Θ, it is possible to calculate $\hat{N}_e$ if the mutation rate of one of the loci used in the analysis is known. Details pertaining to our use of a fast mtDNA mutation rate (3.21×10^{-9} subs/year) based on 0.8% divergence per million years between Western Atlantic and Eastern Pacific *S. lewini* lineages after closure of the Panamanian Isthmus (Duncan et al 2006), and a slow rate (4.0×10^{-9} subs/year) based on the first appearance of *Sphyrna* in the fossil record 20-23 MYA (Cappetta 1987), are in Nance et al. (*in prep*).

Combining Estimates to Increase Precision

Increased precision may be achieved by combining estimates across different methods and calculating the harmonic mean of $\hat{N}_e$ (Waples 1991). However, the time periods associated with each type of method must be concordant. Single-sample, contemporary estimates are generally not analogous to two-sample estimates (i.e. as in the TM method), because the former considers $\hat{N}_b$ in different years while the latter estimates N_e per generation (Waples 2005). Therefore, we took the weighted harmonic mean of all single-sample $\hat{N}_b$ (using LD, SA, and HE methods) for each site in the two years considered in the TM analyses to get an overall estimate of N_b, and compared this single-sample $\hat{N}_b$ to the two-sample (TM) $\hat{N}_e$. We used the method of Waples and Do (*in press*) to calculate the weighted harmonic mean for each site and combine all years and single-sample methods. Because only the LD method has a specific formula to calculate the variance in $\hat{N}_e$, and this variance is required to calculate appropriate weights to each

estimate prior to taking the harmonic mean, we assigned weights to each estimate based on the known performance of the single-sample methods, as recommended by Waples (*pers. comm.*). Because both the SA and LD methods have been shown to yield similarly precise estimates in simulation studies, while the HE method has been shown to be much less precise, we use weights of 0.5 for both SA and LD estimates of N_e and a weight of 0.1 for the HE estimates when calculating the weighted harmonic mean for each site.

Although the time period addressed with coalescent methods (MSVAR and IMa) is the same, and both programs operated under similar assumptions (i.e. change in population size, SSM model for microsatellite evolution), we did not calculate the harmonic mean of their respective estimates because there are several inherent differences between the models used in coalescent simulations (namely, a population closed to migration in MSVAR and populations open to gene flow in IMa). Also, estimates from both methods rely heavily on the mutation rate of the markers, and MSVAR included only microsatellite loci, while IMa included rate information on mtDNA.

Results

Microsatellite and MtDNA Diversity and Neutrality

Three loci in only two of ten samples deviated from Hardy-Weinberg expectations after sequential Bonferroni correction of α (Rice 1989) and another locus deviated from HWE in one of ten samples; see Supplementary Material of Nance et al. (*in prep*). After sequential Bonferroni correction, tests for linkage disequilibrium (LD)

revealed two loci (Cli-12 and Cli-100) in LD, but in samples from only two locations. Micro-Checker revealed five loci had no nulls in any samples and 10 loci had potential nulls in one or two samples.

We found seven haplotypes for the 548 portion of mtDNA control region. Details regarding their distribution throughout the EP region can be found in Nance et al. (*in prep*), but nucleotide and haplotype diversities are shown here in Table 3.1. All values of Tajima's *D* and Fu's F_s were positive for each sample, though none were significant (Table 3.1).

Cohort Divisions per Population

After assigning each individual in our sampled populations to a specific age class, our sample sizes decreased in all cases (see Table 3.1). We analyzed those cohorts with the highest number of individuals. Our samples collected from La Paz, Mexico in 2007 and from Santa Catalina, Panama had no data regarding total length for any of individuals. Tissue from these samples was obtained from recently discarded or frozen heads of juveniles, with no data on body length or sex available. However, since all individuals from these samples were juveniles, we opted to analyze them as one single cohort rather than omit them from the analysis. Therefore, results from these samples must be interpreted with caution.

Contemporary Methods

Linkage Disequilibrium (LD) Method. With the LD method, we obtained non-infinite point estimates of N_e for little over half of our 13 cohorts (see Table 3.2). Only two of those point estimates had a 95% confidence interval without an infinite upper boundary. Combined cohorts from each site yielded $\hat{N}_b$ that was generally larger than estimates from individual cohorts in a given year (with the exception of infinite $\hat{N}_b$, and $\hat{N}_b$ for the combined cohorts from Tarcoles, Costa Rica). The P_{crit} values for the inclusion of low-frequency alleles for each cohort analyzed can be seen in Table 3.2.

Sibship Assignment (SA) Method. For all cohorts analyzed, point estimates of N_e were less than 100 individuals. 95% confidence intervals for these point estimates were much narrower than for the LD method (Table 3.2). Only two cohorts had upper CI boundaries nearing infinity (Mazatlan 2006 and Manta 2007).

Heterozygote Excess (HE) Method. This method proved to be both the most consistent and least precise of all single-sample methods we used. All point estimates of N_e were infinite, with 95% confidence intervals ranging from zero to infinity for all cohorts (Table 3.2).

Temporal Method (TM). Our calculations in MATLAB resulted in a correction factor of C = 63.5, and a mean generation length of G = 21.76 years. Estimates of genetic drift across years (F_s') generated in TempoFs are shown in Table 3.3. For all pairs of cohorts, $\hat{N}_e$ were less than 100 individuals, except for those from Manta, Ecuador.

Coalescent-Based Methods

MSVAR. Point estimates of current N_e based on a coalescent approach using only microsatellite data were generally higher than those based on contemporary methods (Table 3.4). Populations from Santa Catalina, Panama and Manta, Ecuador were over twice as large as those from the other EP sites analyzed. While the 95% highest probability densities (HPDs) for these point estimates were broad, none were infinite (Table 3.4). Point estimates of ancestral N_e were all at least two orders of magnitude greater than current $\hat{N}_e$, with similarly broad, though not infinite 95% HPDs (Table 3.4). Likewise, 95% HPDs of the time since population declines initiated are also broad (Table 3.4).

IMa. As with MSVAR, IMa suggested a two-order magnitude decline in current $\hat{N}_e$ with respect to ancestral $\hat{N}_e$ for most populations (Table 3.5). The exception to this general trend was Cebaco Island (CEB), which had a current $\hat{N}_e$ of 132,754 (calculated with the slower, fossil-calibrated μ) and 104,870 (calculated with the faster, Isthmus μ), and a slightly larger ancestral $\hat{N}_e$ of 449,830 (fossil) and 355,345 (Isthmus). This larger estimate for CEB was apparent only when analyzed with Santa Catalina; analyses of CEB with Manta suggested current $\hat{N}_e$ = 3487 (fossil), 2754 (Isthmus), respectively (Table 3.5).

Harmonic Means of Estimates

Weighted harmonic means of $\hat{N}_b$ per site are shown in Table 3.3. All means are smaller than 100, with the exception of Cebaco Island (CEB). The weighted harmonic

mean of $\hat{N}_{b\,(CEB)}$, however, is based on estimates from only one year while the others are based on estimates across two years.

Discussion

Overall, current N_e estimated using contemporary methods is within the range of current estimates based on long-term genetic variation and the coalescent process (particularly when the relationship $N_b \leq N_e \leq N_b$ x G, where G is the mean generation time [Wang 2009], is considered). In addition to this agreement among contemporary and coalescent methods for current N_e, both MSVAR and IMa suggested that ancestral N_e was much larger (at least two orders of magnitude) than current N_e (Tables 3.4 and 3.5). Furthermore, 95% HPDs of the timing of population decline throughout the EP, as estimated in MSVAR, suggested that the onset of decline was as early as a few centuries ago (Table 3.4). That contemporary methods based on genetic change across generations are in such close agreement with coalescent methods based on genetic diversity (Θ) and long-term N_e, suggest a sudden population decline and a relatively stable (smaller) population size since the onset of decline.

Precision and Bias in Estimates

Despite non-infinite point estimates of contemporary N_e being in close agreement with coalescent estimates, all contemporary estimates suffered from small sample size (s). This affected precision and bias to varying degrees across all contemporary methods used. Not surprisingly, the HE method consistently resulted in infinite point estimates

and 95% confidence intervals (CIs). Such imprecise results using this method were also found by Luikart and Cornuet (1999), Nomura (2008), and Wang (2009). Imprecision arises because the average excess in heterozygosity is inversely proportional to true N_e, so as true N_e increases, precision in estimating it based on excess heterozygosity decreases (Wang 2005). Additionally, violation of the assumption of random mating can lead to overestimates of drift (Wang 2005). Unequal sex ratios and reproductive skew can strongly affect genetic estimates of N_e (Schmeller and Merilä 2007) and despite *S. lewini* litters being comprised of equal proportions of males and females (Liu and Chen 1999; Torres-Huerta 1999, Bejarano-Álvarez 2007), these proportions change as the sharks mature and pregnant females frequent shallow coastal areas for pupping (Klimley 1987; Stevens and Lyle 1989; Torres-Huerta 1999; Bejarano-Alvarez 2007; Martinez-Ortiz et al. 2007; Zanella 2008). Such behavior may make females more susceptible to fishing pressures, particularly artisanal fishers who typically set drift nets along coastal pupping grounds. We can not be certain that this behavior results in skewed adult sex ratios (nearly all of our samples were from juveniles, so we can't directly estimate this), however it should be considered in further analyses and perhaps conservation planning.

As with the HE method, the cause for infinite point estimates for six of 13 cohorts using the LD method is small s relative to true N_e, leading to a weak signal of genetic drift. Although low to moderate and high migration can bias estimates downward and upwards, respectively (Waples and Do *in press*), this likely had little effect on our estimates as migration rates between EP populations are low (Nance et al. *in prep*). Additionally, the LD method is robust to populations that are not ideal (Waples 2006), so

violations of this assumption likely had little effect on our estimates relative to the effect of small s.

All point estimates of N_e under the SA method were much lower than estimates from other methods ($\hat{N}_e$ < 100 individuals) and had much narrower CIs (Table 2). However, simulated data suggest that the SA method will downwardly bias $\hat{N}_e$ when true N_e is large and sample sizes are less than 50 individuals due to erroneous sibship assignments between cousins or unrelated individuals (Wang 2009). Additionally, because the distribution of $\hat{N}_e$ is so skewed, CIs are much tighter if $\hat{N}_e$ is low, so the downward bias in $\hat{N}_e$ leads to false greater precision (Waples and Do *in press*).

As with the other methods, low precision and greater bias affect TM estimates when sample sizes consist of less than 50 individuals (Waples and Yokota 2007). This method is also sensitive to the time between samples, and even though we applied the Jorde and Ryman (1995) correction for overlapping generations, all samples were only one year apart. Precision is increased and bias decreased when samples span five or more generations (Waples and Yokota 2007). Since generation time in *S. lewini* is roughly 22 years, our TM estimates of N_e likely represent $\hat{N}_b$ as we did not sample two generations but rather two cohorts from the same generation.

Despite the fact that all TM estimates of N_e were larger than the weighted harmonic mean of all single-sample $\hat{N}_b$ (Table 3) as expected, the downward bias in SA estimates of N_b probably led to a downward bias in the harmonic mean of $\hat{N}_b$. Since $\hat{N}_b$ varied between years in most cases however, the harmonic mean is still useful by

providing the average number of breeders per site per year. Inter-annual differences in $\hat{N}_b$ may reflect fluctuating population size, caused by unequal sex ratios (as mentioned above), nonrandom variation in reproductive success (Waples 2002), or indicate that a different population of breeding adults contributes to consecutive cohorts.

Long-Term Estimates of N_e

Unlike the contemporary methods, coalescent approaches estimate a long-term value of current N_e and ancestral N_e, so they aren't sensitive to genetic change across one generation but rather model the genealogical histories of the loci through deeper (evolutionary) time. For that reason, our sample sizes were larger and consisted of all individuals sampled from a population, and estimates reflect long-term N_e, rather than N_b or N_e at the time of sampling. There was overlap among the 95% HPDs between estimates from MSVAR and IMa (see Tables 3.4 and 3.5), though the HPDs from IMa were wider. This increased uncertainty is probably caused by the more complicated model implemented in IMa (i.e. isolation and divergence of an ancestral population with current gene flow), whereas MSVAR models only change in N_e for a single population.

Regarding the increased uncertainty of our IMa results, a recent analysis of violation to the Isolation with Migration model assumptions implemented in IMa suggests that both ancestral population size and 95% HPDs will be biased upwards when there is gene flow from an unsampled population (Strasburg and Rieseberg 2009). We have previously shown demographically low but evolutionarily significant levels of

migration between all EP populations (Nance et al. *in prep*), however these rates of migration are low enough to not severely bias estimates (Strasburg and Rieseberg 2009).

As pointed out in the results, the IMa estimate of current N_e for the Cebaco Island (CEB) population is much larger than all other current estimates of N_e (Table 3.5). However, we feel this is due to a poor estimate of Θ_{CEB} in this particular pairwise analysis, and Θ is used to calculate $\hat{N}_e$. When paired with Santa Catalina (SCA), the posterior probability density of Θ_{CEB} would consistently plateau at a large value and remain there, regardless of the prior used. However, because Θ_{CEB} did not behave this way when analyzed with Tarcoles and Manta, we do not consider the associated high estimate of $N_{e\ CEB}$ to be accurate.

Implications for Conservation

Despite having what appears to be a data set sufficient to meet the criteria for reliable, precise estimates of N_e using genetic methods (i.e. samples from multiple years, life history data to apply appropriate corrections for multiple generations, and 15 highly polymorphic microsatellite loci), our estimates based on most of the contemporary methods still suffered from small sample sizes, particularly after we corrected for age-specific cohorts. This will likely be a common problem with large, pelagic marine fishes with overlapping generations, where collecting a hundred or more samples is difficult.

What we can conclude is that EP populations of *S. lewini* are likely not very small (<200), because our sample sizes of <50 were insufficient to yield precise estimates of N_e (Waples and Do *in press*). However, our estimates of N_e are much lower than the

estimated 7000 *S. lewini* born each year in a Hawaii nursery based on tag-recapture data (Clarke 1971; Duncan and Holland 2006). At this site though, juvenile mortality due to natural causes and fishing is quite high (0.85 – 0.93) in the first year of life (Duncan and Holland 2006). Our estimates of N_e suggest juvenile mortality may be similarly high in the EP, and perhaps provide an example of the low N_e:N_c (census N) evident in several marine species (Hedgecock 1994, Hauser et al. 2002, Allendorf et al. 2008).

Although several contemporary estimates suffered from low precision, non-infinite point estimates were similar to coalescent-based estimates, when considering the relationship between N_b and N_e. Furthermore, even when point estimates and upper CIs are infinite, finite lower CI limits can provide reasonable boundaries to N_e (Waples and Do *in press*). Also, what contemporary estimates may lack in precision, they make up for in biological information regarding reproductive behavior. That contemporary estimates vary considerably across some years suggests discrete breeding populations use the same nurseries. However, until we can attain more confidence in our contemporary estimates, and increase sample sizes, we can not make these biological inferences with confidence.

The magnitude of decline evident in comparisons between current and ancestral $\hat{N}_e$ warrants attention from a conservation standpoint, considering *S. lewini* is listed as endangered globally (IUCN 2007) yet still heavily fished throughout its EP range (Dulvy et al. 2008). However, the most striking result from our analyses is the close overlap between contemporary and coalescent-based estimates. This overlap suggests population decline occurred rapidly, and the much smaller, post-reduction population size has been relatively constant since.

Literature Cited

Anislado-Tolentino, V. and C. Robinson-Mendoza. 2001. Age and growth for the scalloped hammerhead shark, *Sphyrna lewini* (Griffith and Smith, 1843) along the central Pacific coast of Mexico. Cien. Mar. 27:501-520.

Anislado-Tolentino, V., M. G. Cabello, F. A. Linares, C. R. Mendoza. 2008. Age and growth of the scalloped hammerhead shark, *Sphyrna lewini* (Griffith and Smith, 1843) from the southern coast of Sinaloa, Mexico. Hidrobiolo'gica 18:31-40.

Beaumont, M. A. 1999. Detecting population expansion and decline using microsatellites. Genetics 153:2013-2029.

Beaumont, M. A. 2003. Conservation genetics. In: Handbook of Statistical Genetics, 2nd edn. (eds. Balding, DJ, Bishop, M, Cannings, C), pp. 751-766. John Wiley & Sons, Chrichester, UK.

Bejarano-Álvarez, M. 2007. Biología reproductiva del tiburón martillo *Sphyrna lewini* (Griffith y Smith, 1834) en Salina Cruz, Oaxaca, México. Tesis de M. en C., Instituto Politecnico Nacional, Centro Interdisciplimatio de Ciencias Marinas, La Paz, 73 pp.

Bonfil, R., Amorim, A. and Simpfendorfer, C. 2005. Southwest Atlantic. In: Fowler, S. L., Cavanagh, R. D., Camhi, M., Burgess, G. H., Cailliet, G. M., Fordham, S. V., Simpfendorfer, C. A. and Musick, J. A. (eds), Sharks, Rays and Chimaeras: The Status of the Chondrichthyan Fishes. Status Survey. , pp. 131-139. IUCN/ SSC Shark Specialist Group, IUCN, Gland, Switzerland and Cambridge, UK.

Branstetter, S. 1987. Age, growth and reproductive biology of the silky shark, *Carcharhinus falciformis*, and the scalloped hammerhead, *Sphyrna lewini*, from the northwestern Gulf of Mexico. Enviro. Biol. Fish. 19:161-173.

Campana, S. E., L. J. Natanson, and S. Myklevoll. 2002. Bomb dating and age determination of large pelagic sharks. Can. J. Fish. Aquat. Sci. 59:450-455.

Cappetta, H. 1987. Chondrichthes II. Mesozoic and Cenozoic Elasmobranchii; vol. 3B. Handbook Paleoichthyology. Fisher. Stuttgart.

Castro, J. I. 1993. The shark nursery of Bulls Bay, South Carolina, with a review of the shark nurseries of the southeastern coast of the United States. Environ. Biol. Fish. 38:37-48.

Castro, J. I. 2009. Observations on the reproductive cycles of some viviparous North American sharks. Aqua: International Journal of Ichthyology 15:205-222.

Chen, C. T., T. C. Leu, S. J. Joung, and N. C. H. Lo. 1990. Age and growth of the scalloped hammerhead, *Sphyrna lewini*, in Northeastern Taiwan waters. Pac. Sci. 44:156-170.

Clarke, TA. 1971. The ecology of the scalloped hammerhead shark, *Sphyrna lewini*, in Hawaii. Pacific Science 25: 133-144.

Clarke, SC, JE Magnussen, DL Abercrombie, MK McAllister, and MS Shivji. 2006. Identification of shark species composition and proportion in the Hong Kong shark fin market based on molecular genetics and trade records. Conservation Biology 20: 201-211.

Compagno, L. J. V. 1984. FAO Species catalogue. vol. 4, parts 1 & 2, Sharks of the World. FAO Fisheries Synopsis, p. 125.

Conrath, C. C., J. J. Gelsleichter, and J. A. Musick. 2002. Age and growth of the smooth dogfish (*Mustelus canis*) in the northwest Atlantic Ocean. Fish. Bull. 100:674-682.

Cortés , E., Arocha, F. Beerkircher, L., Carvalho, F., Domingo, A., Heupel, M., Holtzhausen, H. Santos M., Ribera M., and Simpfendorfer, C. 2009. Ecological risk assessment of pelagic sharks caught in Atlantic pelagic longline fisheries. Aquatic Living Resources 22 (in press).

De Bruyn, P., S. F. J. Dudley, G. Cliff, and M. J. Smale. 2005. Sharks caught in the protective gill nets off KwaZulu-Natal, South Africa. 11. The scalloped hammerhead shatk, *Sphyrna lewini* (Griffith and Smith). Afr. J. Mar. Sci. 27:517-528.

Dudley, S and C Simpfendorfer. 2006. Population status of 14 shark species caught in the protective gillnets off KwaZulu-Natal beaches, South Africa, 1978-2003. Marine and Freshwater Research 57: 225-240.

Dulvy, N.K., J. K. Baum, S. Clarke, L. J. V. Compagno, E. Cortés, A. Domingo, S.Fordham, S. Fowler, M. P. Francis, C. Gibson, J. Martínez, J.A. Musick, A. Soldo, J. D. Stevens, and S. Valenti. 2008. You can swim but you can't hide: the global status and conservation of oceanic pelagic sharks and rays. Aquatic Conservation: Mar.Fresh. Ecosys. 18: 459-482.

Duncan, K. M., and K. N. Holland. 2006. Habitat use, growth rates and dispersal patterns of juvenile scalloped hammerhead sharks Sphyrna lewini in a nursery habitat. Mar. Ecol. Prog. Ser. 312:211-221.

Duncan, K. M., A. P. Martin, B. W. Bowen, H. G. De Couet. 2006. Global phylogeography of the scalloped hammerhead shark (*Sphyrna lewini*). Mol. Ecol. 15:2239-2251.

Excoffier, L., G. Laval, and S. Schneider. 2005. Arlequin ver. 3.0: An integrated software package for population genetics data analysis. Evol. Bioinformatics Online 1:47-50.

Felsenstein. J. 1971. Inbreeding and variance effective numbers in populations with overlapping generations. Genetics 68:581-597.

Fu, Y. X. 1996. New statistical tests of neutrality for DNA samples from a population. Genetics. 143:557-570.

Gomez-Uchida, D. and M. A. Banks. 2006. Estimate of effective population size for the long-lived darkblotched rockfish *Sebastes crameri*. J. Heredity 97:603-606.

Hansen, M. M., E. E. Nielsen, D. E. Ruzzante, C. Bouza and K. D. Mensberg. 2000. Genetic monitoring of supportive breeding in brown trout (Salmo trutta L.), using microsatellite DNA markers. Can. J. Fish. Aquat. Sci. 57:2130-2139.

Hazin, F., A. Fischer, and M. Broadhurst. 2001. Aspects of reproductive biology of the scalloped hammerhead shark, *Sphyrna lewini*, off northeastern Brazil. Enviro. Biol. Fish. 61:51-159.

Hey, J., and R. Nielsen. 2004. Multilocus methods for estimating population sizes, migration rates and divergence time, with applications to the divergence of *Drosophila pseudoobscura* and *D. persimilis*. Genetics 167:747-760.

Hill, W. G. 1981. Estimation of effective population size from data on linkage disequilibrium. Gen. Res. 38:209-216.

Jorde, P. E. and N. Ryman. 1995. Temporal allele frequency change and estimation of effective size in populations with overlapping generations. Genetics 139:1077 1090.

Jorde, P. E. and N. Ryman. 2007. Unbiased estimator for genetic drift and effective population size. Genetics 177:927-935.

Klimley, A. P. 1981. Grouping behavior in the scalloped hammerhead. Oceanus 24:65 71.

Klimley, A. P. 1987. The determinants of sexual segregation in the scalloped hammerhead shark, *Sphyrna lewini*. Enviro. Biol. .Fish. 18:27-40.

Krimbas, C. B. and S. Tsakas. 1971. The genetics of *Dacus oleae*. V. Changes of esterase polymorphism in a natural population following insecticide control – selection or drift? Evolution 25:454-460.

Laurie-Ahlberg, C. and B. S. Weir. 1979. Allozyme variation and linkage disequilibrium in some laboratory populations of *Drosophila melanogaster*. Genet. Res. 32:215 229.

Liu, K. M. and C. T. Chen. 1999. Demographic analysis of the scalloped hammerhead, *Sphyrna lewini*, in the Northwestern Pacific. Fish. Sci. 65:218-223.

Luikart, G. and J. M. Cornuet. 1999. Estimating the effective number of breeders from heterozygote excess in progeny. Genetics 151:1211-1216.

Martínez-Ortíz, J., F. Galván-Magaña, M. Carrera-Fernández, D. Mendoza-Intriago, C. Estupiñán-Montaño, and L. Cedeño-Figueroa. 2007. Abundancia estacional de tiburones desembarcados en Manta – Ecuador. pp. 9-27 in: Tiburones en el Ecuador: Casos de estudio. (Eds. J. Martínez-Ortíz, F. Galván-Magaña) EPESPO-PMCR. Manta, Ecuador.

Musick, J. A., G. Burgess, G. Cailliet, M. Camhi, and S. Fordham. 2000. Management of sharks and their relatives (Elasmobranchii). Fisheries 25:9-13.

Nance, H. A., T. S. Daly-Engel, and P. B. Marko. 2009. New microsatellite loci for the endangered scalloped hammerhead shark, *Sphyrna lewini*. Mol. Ecol. Res. 9:955-957.

Nance, H. A., A. P. Klimley, F. Galván-Magaña, J. Martínez-Ortíz, and P. B. Marko. *in prep*. Decline and divergence: Evolutionary processes causing population structure in the scalloped hammerhead shark, *Sphyrna lewini*, in the Eastern Pacific.

Nei, M and F. Tajima. 1981. Genetic drift and estimation of effective population size. Genetics 98:625-640.

Nomura, T. 2008. Estimation of effective number of breeders from molecular coancestry of single cohort sample. Evol. App. 1:462-474.

Piercy, A. N., J. K. Carlson, J. A. Sulikowski, and G. H. Burgess. 2007. Age and growth of the scalloped hammerhead shark, *Sphyrna lewini*, in the north-west Atlantic Ocean and Gulf of Mexico. Mar. Fresh. Res. 58:34-40.

Pratt, H., Jr., and J. G. Casey. 1983. Age and growth of the shortfin mako, *Isurus oxyrinchus*, using four methods. Can. J. Fish. Aquat. Sci. 40:1944-1957.

Pudovkin, A. I., D. V. Zaykin and D. Hedgecock. 1996. On the potential for estimating the potential number of breeders from heterozygote-excess in progeny. Genetics 144:383-387.

Rice, W. R. 1989. Analyzing tables of statistical tests. Evolution 43:223-225.

Robertson, A. 1965. The interpretation of genotypic ratios in domestic animal populations. Animal Production 7:319-324.

Schmeller, D. S. and J. Merilä. 2007. Demographic and genetic estimates of effective population and breeding size in the amphibian *Rana temporaria*. Conserv. Biol. 21:142-151.

Strasburg, J. L., and L. H. Rieseberg. 2009. How robust are "Isolation with Migration" analyses to violations of the IM model? A simulation study. Mol. Biol. Evol. (in press).

Stevens, J. D. and J. M. Lyle. 1989. Biology of three hammerhead shatks (*Eusphyra blochii*, *Sphyrna mokarran*, and *S. lewini*) from Nothern Australia. Aust. J. Freshwater Res. 40:129-146.

Sulikowski, J. A., J. Kneebone, S. Elzey, J. Jurek, P. Danley, W. H. Howell, and P. C. W. Tsang. 2005. Age and growth estimates of the thorny skate (*Amblyraja radiate*) in the western Gulf of Maine. Fish. Bull. 103:161-168.

Tajima, F. 1989. The effect of change in population size on DNA polymorphism. Genetics. 123: 597-601.

Torres-Huerta, A. M. 1999. Observaciones sobre la biologia reproductive de la Cornuda Barrosa, *Sphyrna lewini* (Griffith y Smith, 1834) (Pisces: Sphyrnidae) en agues del noroeste de Mexico. Tesis de M. en C., UNAM Escuela Nacional de Estudios Profesionales, Iztacala. 68 pp.

Turner, T. F., P. Wares and J. R. Gold. 2002. Genetic effective size is three orders of magnitude smaller than adult census size in an abundant, estuarine-dependent marine fish (*Sciaenops ocellatus*). Genetics 162:1329-1339.

Van Oosterhout, C., W. F. Hutchinson, D. P. M. Wills, and P. Shipley. 2004. Micro Checker: software for identifying and correcting genotyping errors in microsatellite data. Mol. Ecol. Notes 4:535-538.

Wang, J. 2004. Sibship reconstruction from genetic data with typing errors. Genetics 166:1963-1979.

Wang, J. 2005. Estimation of effective population sizes from data on genetic markers. Philos. Trans. R. Soc. Ser. B 360:1395-1409.

Wang, J. 2009. A new method for estimating effective population sizes from a single sample of multilocus genotypes. Mol. Ecol. 18:2148-2164.

Waples, R. S. 1989. A generalized approach for estimating effective population size from temporal changes in allele frequency. Genetics 121:379-391.

Waples, R. S. 1990. Conservation genetics of Pacific salmon. III. Estimating effective population size. J. Hered. 81:277-289.

Waples, R. S. 1991. Genetic methods for estimating the effective size of cetacean populations. Rep. Int. Whal. Comm. (Special Issue 13):279-300.

Waples, R. S. 2002. Effective size of fluctuating salmon populations. Genetics 161:782 791.

Waples, R. S. 2005. Genetic estimates of contemporary effective population size: To what time periods do estimates apply? Mol. Ecol. 14:3335-3352.

Waples, R. S. 2006. A bias correction for estimates of effective population size based on linkage disequilibrium at unlinked gene loci. Conserv. Gen. 7:167-184.

Waples, R. S. and M. Yokota. 2007. Temporal estimates of effective population size in species with overlapping generations. Genetics 175:219-233.

Waples, R. S. and C. Do. 2008. LDNE: a program for estimating effective population size from data on linkage disequilibrium. Mol. Ecol. Res. 8:753-756.

Waples, R. S. and C. Do. Linkage disequilibrium estimates of contemporary N_e using highly variable genetic markers: A largely untapped resource for applied conservation and evolution. Evol. App. *in press*.

Zanella, I. 2008. Caracterización de la pesca y algunos aspectos sobre la reproducción, alimentación y distribución del tiburón martillo *Sphyrna lewini*, Sphyrnidae, en el Pacífico de Costa Rica. Tesis de M. en C., Universidad Nacional de Costa Rica, Instituto Internacional de Conservation y Manejo de Vida Silvestre, 104 pp.

Zeeberg, J, A Corten, and ED Graff. 2006. Bycatch and release of pelagic megafauna in industrial trawler fisheries off Northwest Africa. Fisheries Research 78: 186-195.

Table 3.1. Number of *Sphyrna lewini* samples genotyped (n_{msat}), numbers of samples used in all contemporary N_e methods after dividing individuals into age-specific cohorts (n_c), and number of samples sequenced (n_{mtDNA}) per year per populaton. Samples LAP 2007 and SCA 2007/08 are noted with as asterisk (*) to note that these three samples had no accompanying data from which we could assign individuals to age-specific cohorts, so we analyzed all samples. Site abbreviations correspond to locations in Figure 1. Location (longitude and latitude), nucleotide (π) and haplotype (h) diversities are shown for mtDNA data. Neutrality statistics Tajima's D, Fu's and F_s; none were significant at $\alpha = 05$. All mtDNA statistics are from previous analyses in Nance et al. (*in prep*).

Population	Coordinates	n_{msat}	n_c	n_{mtDNA}[1]	π	h	D	F_s
La Paz, Mexico (LAP) 2001	N 24.20, W 110.40	31	15					
LAP2004		22	22					
LAP2006		30	27					
LAP2007*		24	24	17	0.001	0.441	12.496	5.435
Mazatlan, Mexico (MAZ)2006	N 23.20, W 106.40	14	20					
MAZ2007		38	32	22	0.001	0.416	11.446	5.709
Tarcoles, Costa Rica (TAR)2007	N 9.80, W 84.80	40	36	20	0.001	0.616	8.730	4.087
TAR2008		40	28					
Santa Catalina, Panama (SCA) 2007*	N 7.56, W 81.30	46	46	22	0.001	0.597	6.509	2.869
SCA2008*		46	46					
Cebaco Island, Panama (CEB) 2008	N 7.55, W 81.00	21	21	18	0.001	0.569	9.012	4.196
Manta, Ecuador (MAN) 2007	S 1.10, 84.95	36	23	20	0.001	0.526	15.064	6.643
MAN2008		43	15					

[1]All mtDNA samples from 2007 except CEB and GPA

Table 3.2. Point estimates of N_b and their associated 95% confidence intervals (CIs) for all three single-sample contemporary methods: Linkage Disequilibrium (LD), Sibship Assignment (SA), and Heterozygote Excess (HE). We have shown the upper limit of the point estimate of N_b for the LD and SA methods, calculated as $\hat{N}_b$ x G. This is based on the relationship $N_b \leq N_e \leq N_b$ x G, where G is a generation time of 21.76 years. We have shown this upper limit so results from coalescent based methods (that actually estimate N_e) are comparable. n_c refers to the number of samples in each cohort analyzed. Under the LD method, P_{crit} refers to the cut-off value we used in including low-frequency alleles, using the rules of Waples and Do (in press).

		LD				SA			HE	
Cohort	n_c	N_b	95% CI	N_b x G	P_{crit}	N_b	95% CI	N_b x G	N_b	95% CI
LAP2001	15	885.6	55.3 - ∞	19270.7	0.05	60	29 - 273	1305.6	∞	0 - ∞
LAP2004	22	∞	1476.5 - ∞	∞	0.03	60	34-153	1305.6	∞	0 - ∞
LAP2006	27	∞	486.4 - ∞	∞	0.02	48	27-87	1044.5	∞	0 - ∞
LAP2007	24	261.2	69.8 - ∞	5683.7	0.03	48	26-94	1044.5	∞	0 - ∞
MAZ2006	14	∞	94.8 - ∞	∞	0.03	76	35 - ∞	1653.8	∞	0 - ∞
MAZ2007	32	92.4	58.3 - ∞	2010.6	0.02	54	33-98	1175.0	∞	0 - ∞
TAR2007	36	418.2	146.7 - ∞	9100.0	0.02	53	34 - 89	1153.3	∞	0 - ∞
TAR2008	28	318.9	109.6 - ∞	6939.3	0.02	46	27 - 82	1001.0	∞	0 - ∞
SCA2007	46	191	110.0 - ∞	4156.2	0.02	50	31 - 84	1088.0	∞	0 - ∞
SCA2008	46	∞	2287.0 - ∞	∞	0.02	66	43 - 106	1436.2	∞	0 - ∞
CEB2008	21	∞	143.3 - ∞	∞	0.03	52	30 - 103	1131.5	∞	0 - ∞
MAN2007	23	420.5	66.6 - ∞	9150.1	0.03	40	21 - 89	870.4	∞	0 - ∞
MAN2008	15	∞	456.9 - ∞	∞	0.04	87	37 - ∞	1893.1	∞	0 - ∞

Table 3.3. $\hat{N}_e$ based on the two-sample contemporary Temporal Method (TM). n_c refers to the numbers of individuals per year in each age-specific cohort analyzed. F' is the amount of genetic drift between successive cohorts estimated in TempoFS (Jorde and Ryman, 2007). Ne represents the effective population size estimate of the generation at the time of sampling, based on the TM method. Mean Nb is the weighted harmonic mean (calculated according to Waples and Do, *in press*) of all single-sample contemporary methods (LD, SA, and HE).

Cohorts	n_c	Fs'	N_e	Mean N_b
LAP2006/2007	27/24	0.025	57.933	43.96
MAZ2006/2007	20/32	0.032	45.223	47.06
TAR2007/2008	36/28	0.015	96.034	43.35
SCA2007/2008	46/46	0.016	89.909	49.52
MAN2007/2008	23/15	0.002	622.483	51.45

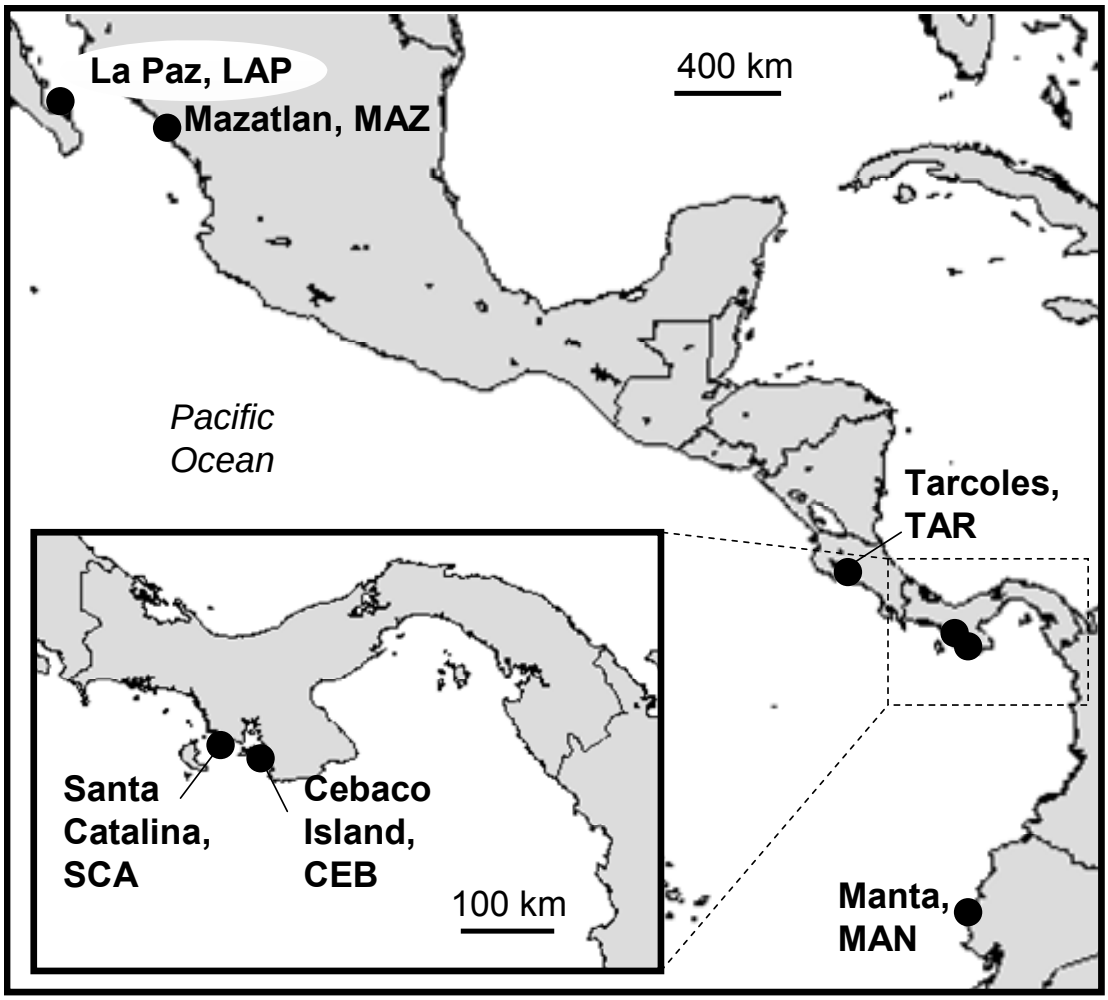
Table 3.4. Current and ancestral $\hat{N}_e$ based on the coalescent method implemented with MSVAR (Beaumont, 1999). $\hat{N}_0$ is the estimated current effective population size, while $\hat{N}_1$ is the estimated ancestral effective size. Time (t) in years indicates the age of onset of decline. For all estimates, 95% highest probability densities (HPDs) reflect the area along the x-axis where 95% of the data lie in plots of posterior probabilities. Analyses in MSVAR were done previously by Nance et al. (*in prep*).

Population	N_{e0}	N_{e1}	t (in years)
LAP	435.51	39627.80	8452.79
95% HPD	36.16 - 4717.37	4718.46 - 324041.03	493.06 - 117733.49
MAZ	384.68	43551.19	6181.59
95% HPD	28.89 - 4627.01	4927.20 - 365426.47	386.99 - 81320.49
TAR	481.95	34994.52	5766.34
95% HPD	49.57 - 4607.87	4102.99 - 289867.82	347.46 - 86616.37
SCA	1003.46	54175.13	15808.84
95% HPD	123.68 - 8150.80	5931.98 - 466874.33	661.46 - 220140.53
CEB	226.67	38256.04	3639.15
95% HPD	8.00 - 4952.22	4463.75 - 333042.76	116.33 - 79031.46
MAN	1015.08	35318.32	16722.45
95% HPD	121.03 - 8483.99	4131.43 - 301647.69	987.87 - 229034.02

Table 3.5. $\hat{N}_e$ based on coalescent methods implemented in IMa (Hey and Neilson, 2004). 95% HPD represents the interval on the x-axis where 95% of the area under the posterior probability density curve lies. N_{e1} refers to the first population in a pair, N_{e2} refers to the second. N_{eA} refers to the ancestral population from which populations 1 and 2 arose. Values of μ for each estimate correspond to a mutation rate of 3.21 x 10^{-9} subs/year calibrated using the first appearance of *Sphyrna* in the fossil record, roughly 21.5 MYA (Cappetta, 1987), and a rate of 4.00 x 10^{-9} subs/year based on the assumption that Western Atlantic and Eastern Pacific S. lewini split 3.5 MYA after the rise of the Panamanian isthmus (Duncan et al. 2006). Analyses in IMa were done previously by Nance et al. (*in prep*).

Populations	μ	N_{e1}	N_{e2}	N_{eA}
LAP-MAZ	3.21 e^{-9}	252.36	2682.97	581089.55
95% HPD		172.67 - 25275.745	1248.51 - 51720.29	271617.86 - 922438.16
	4.00 e^{-9}	199.35	2119.43	459034.21
95% HPD		136.13 - 19926.70	984.29 - 40774.87	214136.13 - 727225.13
MAZ-TAR	3.21 e^{-9}	345.33	7610.61	594703.65
95% HPD		292.21 - 24678.04	1633.69 - 74711.51	369572.96 - 1130634.24
	4.00 e^{-9}	272.80	6012.04	469788.73
95% HPD		230.37 - 19455.50	1287.96 - 58900.52	291361.26 - 891361.26
TAR-SCA	3.21 e^{-9}	4021.14	8334.48	567176.61
95% HPD		1371.37 - 21360.85	2756.03 - 29187.30	322189.25 - 1007742.11
	4.00 e^{-9}	3176.52	6583.86	448043.62
95% HPD		1081.15 - 16840.31	2172.77 - 23010.47	254005.24 - 794476.44
SCA-CEB	3.21 e^{-9}	302.17	132754.06	449829.72
95% HPD		209.19 - 8716.34	10692.01 - 130363.29	332217.20 - 1004288.77
	4.00 e^{-9}	238.70	104869.64	355344.94
95% HPD		164.92 - 6871.73	8429.32 - 102774.87	261910.99 - 791753.93
CEB-MAN	3.21 e^{-9}	1348.13	3486.54	551669.82
95% HPD		790.28 - 89580.77	1560.64 - 57212.42	382456.54 - 919449.70
	4.00 e^{-9}	1064.96	2754.21	435793.97
95% HPD		623.04 - 70623.04	1230.37 - 45104.71	301518.32 - 724869.11

Figure 3.1. Map of Eastern Pacific range of *Sphyrna lewini* and study area. Sample localities and their associated abbreviations indicated by black dots. The two Panamanian sites are enlarged due to their close proximity to one another.



CHAPTER FOUR

TEMPORAL GENETIC STRUCTURE IN A SCALLOPED HAMMERHEAD SHARK (*SPHYRNA LEWINI*) NURSERY, LA PAZ BAY, MEXICO: IMPLICATIONS FOR PHILOPATRIC BEHAVIOR

Abstract

Philopatric behavior, characterized by site-fidelity to certain 'home' areas for reproduction and/or foraging, is important to identify in species of conservation concern, as such knowledge can help assess their risk of local extinction. Though patterns of spatial genetic structure have been used to infer philopatry, temporal data are required to determine whether the same populations return to a given site year after, and therefore, if spatial genetic patterns persist through time. Accordingly, samples of the endangered scalloped hammerhead shark, *Sphyrna lewini*, were collected across four year (2001, 2004, 2006, and 2007) from a purported nursery in La Paz Bay, Mexico to determine whether individuals are genetically homogenous between years. While F_{ST} values from global AMOVAs indicated that samples from La Paz were generally more similar to each other than to a more distant nursery near Tarcoles, Costa Rica over 3000 km away, our findings were inconsistent with the expected genetic signature of annual philopatry. Pairwise F_{ST} was significant between some, but not all, samples collected in La Paz across years, and between most, but not all, samples from La Paz and Tarcoles. Similarly, levels of relatedness estimated among juveniles both within and between years in La Paz Bay suggested that the same breeding population is not using this site for parturition every year, but may return over longer time intervals. Half-siblings were common across all years, but full-sibling pairs were rare and only occurred within the

same sample year, or across three or more years. This patchy pattern of spatial and temporal differentiation may be caused by *S. lewini* not reproducing annually, by individuals using nurseries other than La Paz Bay, or could result from more complicated evolutionary processes. Overall, these temporal data suggest that multiple breeding stocks are using La Paz Bay for reproduction, which may buffer the effects of local overfishing in this region.

Introduction

Philopatry, literally "home-loving", describes behavior in which animals faithfully remain at or return to their home area (Mayr 1963). In the strictest sense of the word, philopatry refers to natal site fidelity, such as in anadromous salmon that return to the exact stream where they were born to reproduce (e.g. Quinn et al. 1999). In a broader sense, philopatry describes the regular use of breeding and/or foraging grounds and in marine taxa has been applied at varying spatial scales ranging from small lagoons used for breeding (Pratt & Carrier 2001) to stretches of coastline over 100 km in length used regularly for parturition (Keeney et al. 2003). Sex-specific philopatry arises when either males or females have a greater tendency to disperse, and has been shown in marine fishes, reptiles, and mammals (e.g. Baker et al. 1998; Pardini et al. 2001; Bowen et al. 2005).

For taxa that are endangered due to overexploitation or habitat loss, the recognition of philopatric behavior and "home" sites that are critical for the persistence of local populations is paramount to assessing the risk of extirpation and implementing

effective management strategies (Hueter et al. 2004). However, large, highly-mobile fishes capable of traveling vast distances in deep off-shore waters are difficult to physically track across many years to determine whether they are faithful to reproductive and/or foraging areas. For these kinds of taxa, genetic data are widely used to infer philopatric behavior at the population level (e.g. Pardini et al. 2001; Feldheim et al. 2004; Carlsson et al. 2007).

Such data have been applied to the globally-endangered (IUCN 2007) scalloped hammerhead shark, *Sphyrna lewini*, for which philopatry to nursery areas has been suggested given the regular occurrence of pregnant females at protected coastal embayments (e.g. Clarke 1971; Compagno 1984; Castro 1993) and the tendency for juveniles to remain in shallow pupping areas until sexually mature (e.g. Klimley 1987; Stevens & Lyle 1989). Recent genetic support for this hypothesis includes spatial differentiation among mtDNA haplotypes along the Western Atlantic range of *S. lewini* (Chapman et al. 2009), and significant structure among mtDNA haplotypes at much larger scales, between continental margins and oceanic islands (Duncan et al. 2006). Although these patterns of genetic differentiation are in agreement with the expected genetic signature of female philopatry, does a pattern of spatial genetic structure at any locus actually mean that distinct populations persist over time? If philopatry is defined as the tendency for individuals to remain at, or return to, a specific location, then temporal data are necessary to determine the ecological and evolutionary significance of spatially distinct populations. This is how philopatry needs to be assessed, rather than inferred

from a snap-shot of population genetic structure at one point in time that may or may not be ephemeral.

To that end, juvenile *S. lewini* were sampled within La Paz Bay, Mexico, a purported nursery for this semi-pelagic shark (Torres-Huerta 1999), across multiple years to characterize their temporal genetic structure. If philopatry for nursery and/or breeding grounds exists in *S. lewini*, then one would expect a lack of temporal genetic variation at a given pupping site across years. Using 15 microsatellite loci, I characterized the temporal genetic structure among *S. lewini* from La Paz Bay collected in 2001, 2004, 2006, and 2007. I also estimated levels of relatedness in order to detect full- and half-siblings within and between sampling years. Individual sharks within La Paz Bay were further compared with juveniles sampled in 2007 near Tarcoles, Costa Rica, another purported nursery for *S. lewini* (Zanella 2008) located over 3000 km south of La Paz. These comparisons of genetic differentiation were made in order to interpret the relative degree of similarity among individuals in La Paz Bay with respect to individuals at a more distant site.

The use of highly-variable nuclear microsatellites enabled detection of genetic differences across such a brief sampling period, whereas low haplotype diversity among Baja California *S. lewini* mtDNA (Duncan et al. 2006; Nance et al. *in prep*) would have likely made detection of differences across this temporal scale difficult, if not impossible. Though use of only nuclear microsatellites will inhibit detection of sex-specific philopatry, earlier evidence of spatial structure at both mitochondrial and nuclear markers precludes the hypothesis that dispersal is sex-specific (Nance et al. *in review*).

Accordingly, the aim of the current study was to characterize temporal patterns of genetic variation that may result from both male and female site fidelity in *S. lewini*.

Methods

Sample Collection and DNA Extraction

We used 107 *S. lewini* samples collected from artisanal fishers from La Paz (LAP), Baja California Sur, Mexico and 40 samples from the Gulf of Nicoya, Tarcoles, Costa Rica in this study. Samples were collected in La Paz between January and March in 2001, 2004, 2006, and 2007 from local fish camps at El Sauzoso, El Saladito, El Mogote, and camps along El Malecon in downtown La Paz (Figure 4.1). Samples from Tarcoles were collected from fishers in 2007. Though samples were collected from different sites near La Paz, fishermen set nets throughout La Paz Bay. Samples were taken from the anal fin or as plugs of muscle tissue from *S. lewini* specimens. Samples were stored in >90% ethanol. Genomic DNA was isolated from each sample using Proteinase K tissue digests in 2X CTAB, followed by two chloroform/isoamyl alcohol (24:1) extractions and precipitation in ethanol. DNA was dried, re-suspended in 50µL water, and frozen.

Microsatellite Genotyping

Of the 15 microsatellite loci we amplified and scored, 13 loci were developed for *S. lewini* [see Nance et al. (2009) for PCR conditions], and two (Cli-12 and Cli-100) were originally developed for the blacktip shark, *Carcharhinus limbatus* (Keeney & Heist

2003). All PCR reactions were performed using a DNA Engine DYAD Peltier Thermal Cycler (MJ Research Inc.) and visualized on an ABI 3130 (Applied Biosystems, Inc.) automated sequencer at the Clemson University Genomics Institute (CUGI). Individual genotypes were then scored using GeneMapper software (Applied Biosystems, Inc.).

Microsatellite Diversity

Microsatellite loci sometimes have null alleles which fail to amplify in individuals, resulting in an excess of homozygotes. The presence of nulls can inflate F_{ST} and genetic distance estimates among populations (Chapuis & Estoup 2007). Therefore, we first checked all loci for evidence of null alleles using the program Micro-Checker (Van Oosterhout et al. 2004). This program applies a Monte Carlo simulation method to estimate differences in the observed and expected frequencies of homozygote and heterozygote allele sizes based on Hardy-Weinberg equilibrium theory. All loci were also tested for deviations from Hardy-Weinberg equilibrium and linkage disequilibrium using Arlequin v. 3.11 (Excoffier et al. 2005).

Genetic Structure

To characterize the partitioning of genetic differentiation among *S. lewini* in La Paz Bay across years, we calculated global F_{ST} using an Analysis of Molecular Variance (AMOVA) in Arlequin (Excoffier et al. 2005). We then estimated pairwise F_{ST} values for all sample years. We also calculated R_{ST} to determine whether the size of alleles, rather than just their frequencies, contributed to levels of differentiation. To better interpret the

level of structure between years in La Paz Bay, we also performed a global AMOVA and estimated pairwise F_{ST} values with the Tarcoles, Costa Rica population included.

Due to our expectation of high relatedness among our sampled individuals from La Paz Bay, we also estimated global (with an AMOVA) and population specific F_{IS} in Arlequin. F_{IS} is a measure of the deviation in heterozygosity among individuals relative to their subpopulation, and may be indicative of inbreeding (Nei 1977).

Assignment Tests

We used the program STRUCTURE v. 2.3.3 (Hubisz et al. 2009) to infer the number of discrete populations in La Paz Bay both within and among sampling years. To determine the probability of K populations among our samples, we ran the program setting K equal to one through six. Each run assumed prior population information and operated under the model of admixture, which allows for individuals to have a mixed ancestry (Pritchard et al. 2000). Each run for each possible K was repeated six times, three with allele frequencies independent from each other, and then three with frequencies correlated. Each of the six independent runs had a burn-in of 10,000 steps and 100,000 MCMC steps.

Kinship Analyses

All of our samples came from artisanal fishermen who generally set their nets or lines close to shore, so samples were comprised of juveniles, which may remain in their natal pupping areas until reaching sexual maturity (e.g. Klimley 1987; Stevens & Lyle

1989). Determining the proportion of related individuals both within and between sampling years could provide insight regarding the regular use of La Paz Bay as a nursery for breeding adults. We used the program Genalex (Peakall & Smouse 2005) to estimate the relatedness coefficient, *r* (Queller & Goodnight 1989), between all possible pairs of individuals based on our nuclear data. We then calculated the percent of individuals within each sample and between sampling years that were in a sibling pair with an r value ≥0.25 (half-siblings), and ≥0.50 (full-siblings).

We also used the program COLONY2 (Wang 2009) which implements an algorithm that searches for the maximum likelihood configuration of sibship assignments for all individuals in a sample based on their genotypes. This program is unique in that it considers the frequency of errors due to allele dropout (Class I), and due to mutation, PCR error, miscalling, and data entry (Class II) when assigning sibship (Wang 2004). All samples consisted of juveniles, so we had no paternal or maternal information to include. For each sample analyzed, allele frequencies were updated during the course of three long runs with high, full-likelihood precision. We set the frequency of errors to 5%, chose polygamous mating systems for both males and females, and set no prior on the size of sibling groups, as *S. lewini* can have up to 31 pups per litter (Compagno 1984). In simulations, populations under polygamous, maximal sibling mating systems led to an overestimate of the probability of sibship, however, this bias was small (Wang 2009). Therefore, violations of our assumptions of random mating should not strongly affect sibship assignments. With the resulting assignments, we calculated the percent of

individuals in each La Paz sample year individually and across all La Paz sample years combined that were in either a half- or full-sibling pair with ≥95% probability.

Results

Microsatellite Diversity

One locus (SLE053) in LAP2007, and one locus (SLE071) in Tarcoles deviated from Hardy-Weinberg expectations after sequential Bonferroni correction of α (Rice 1989); see Appendix B. After sequential Bonferroni correction, no loci in any samples showed evidence of linkage disequilibrium. Results from Micro-Checker revealed eight loci with potential null alleles, but only two were present in two out of five year classes and the other six were present in one of five (different) year classes tested. As there appeared to be no systematic deviation at any single locus among all years and the Tarcoles sample, we kept all loci in subsequent analyses

Genetic Structure

A global AMOVA revealed no significant differentiation among La Paz sampling years (F_{ST} = 0.002, p > 0.05); see Table 4.1. However, pairwise F_{ST} was significant between La Paz sample years 2004/2006 (F_{ST} = 0.019, p = 0.00) and between 2006/2007 (F_{ST} = 0.008, p = 0.018); see Table 2. Sample LAP2001 did not differ significantly from any other sample year, and samples 2004/2007 were not significantly differentiated from each other. Neither global nor pairwise values of R_{ST} suggested significant differentiation between sampling years, and levels of observed heterozygosity were not

significantly different from expected, as indicated by both a global AMOVA and population specific estimates of F_{IS} (data not shown).

When individuals from Tarcoles were included in the AMOVA, there was significant differentiation with global $F_{ST} = 0.004$ ($p = 0.009$); see Table 4.1. Additionally, all sample years except 2001 from La Paz were significantly differentiated from Tarcoles in pairwise F_{ST} analyses, though the magnitude of structure was less than that between LAP2004/2006 (Table 4.2).

Assignment Tests

All analyses in STRUCTURE resulted in K=1 having the highest probability when all La Paz sampling years were analyzed together, and when years were analyzed individually, indicating no differentiation among or within sampling years. Furthermore, graphic plots of assignment for K=1 – 6 suggested complete admixture among all sampling years (data not shown).

Kinship Analyses

Results from Genalex, which estimated the coefficient of relatedness, r (Queller & Goodnight 1989), revealed that at least half of the individuals in each sample were half-siblings ($r \geq 0.25$), with the exception of LAP2001, which had only 48.39% of individuals being members of a half-sibling pair (see Table 4.3). Only 8.33% of individuals from LAP2007 were members of a full-sib pair ($r \geq 0.50$), and no other years contained any full-sibling members. However, when all years were combined (in an

effort to detect siblings across sampling years), 81.31% and 8.41% of individuals were members of half- and full-sib pairs, respectively.

Analyses in COLONY2 (Wang 2009) revealed similar proportions of full- and half-siblings among sampled individuals in La Paz Bay. With the exception of LAP2004, the majority of individuals in each sample were members of a half-sibling pair, while far fewer individuals were members of full-sibling pairs (see Table 4.3). When all four years were combined, 10.28% of individuals were members of a full-sibling pair containing sharks from different years, whereas 85.05% were members of half-sibling pairs with a likelihood ≥ 95%. As with results using Queller and Goodnight's (1989) relatedness coefficient, *r*, results from COLONY2 indicated that all full-sibling pairs were either from the same year or between three or more years, rather than between consecutive years.

Discussion

General Conclusions

A global AMOVA and results from STRUCTURE suggested that samples collected in La Paz Bay among four years were not significantly differentiated, particularly when compared to the significant differentiation detected in a global AMOVA that included individuals from Tarcoles, Costa Rica. However, pairwise F_{ST} results revealed a more complicated pattern. Sample years 2004/2006 and 2006/2007 from La Paz Bay were significantly distinct (see Table 4.2), while other pairwise sampling years were not. Additionally, all samples from La Paz Bay except 2001

differed significantly from Tarcoles, yet pairwise differences between 2004 and 2006 within La Paz Bay (F_{ST} = 0.019) were greater than those between La Paz Bay and Tarcoles (F_{ST} = 0.006 – 0.010, see Table 4.2), complicating the interpretation of these spatial and temporal differences in *S. lewini*. It seems that, while there is a generally tendency for individuals at a given site between years to be more genetically similar than individuals separated by 3000+ km of coastline, there exists temporal and spatial patchiness in this trend, which is inconsistent with strict annual philopatry to specific nursery areas.

Sibship reconstructions revealed that half-siblings were prevalent both within and between sampling years in La Paz Bay, but full siblings were much rarer (Table 4.3). Those individuals that were full-siblings were either sampled in the same year or separated by three or more years, suggesting that juveniles sharing both parents were either litter mates (within sampling year) or from a population of breeding adults that returned to or remained near La Paz Bay. These data, like the temporal structure data, imply that if *S. lewini* is philopatric to La Paz Bay, the same adults return to this site less frequently then every year. Whether this temporal pattern results from *S. lewini* not reproducing annually, or from adults using different reproductive grounds in different years is unclear.

Explanations for Temporal Variation

Reproductive cycle. Though studies have shown that *S. lewini* is physically able to reproduce annually, with simultaneous development of ovarian follicles and embryos

(Castro 2009), and the noted presence of spermatozoa in gravid females (Hazin et al. 2001; de Bruyn et al. 2005), annual reproduction has not been directly observed (i.e. there are no tagging data showing an individual female has given birth in consecutive years). Therefore, it is possible that not all females reproduce annually, particularly given a 10-12 month gestation period (Stevens & Lyle 1989; Liu & Chen 1999; Hazin et al. 2001) and the high energetic cost of viviparity. Such "low frequency reproduction" has been described in several iteroparous fish, amphibians, and reptiles, when energetically-high activities are associated with reproduction, such as live birth and breeding migrations (Bull & Shine 1979). More recently, this tendency for skipped-reproduction has been found in several species of bony fish, and is thought to be a strategy that ensures higher long-term fitness when energetically-costly reproduction in a given year compromises survival (Rideout et al. 2005). It may be that temporal differences across some, but not all years in La Paz Bay reflect the fact that *S. lewini* does not reproduce every year, and therefore, does not return to this site annually for parturition.

Scale of site-fidelity. An alternative explanation for the temporal genetic variation evident in La Paz Bay is that adults are not returning to the exact same site for breeding or parturition, but rather utilize a larger area for reproduction. This kind of regional philopatry has been observed in blacktip sharks (*Carcharhinus limbatus*), where spatial structure was evident between nurseries along the gulf coasts of Florida, Texas, and the Yucatán peninsula, yet not between sites separated by 100-250 km within Florida's coastline (Keeney et al. 2005). Perhaps *S. lewini* similarly exhibits site fidelity at larger scales such that the entire Gulf of California (GOC) is considered 'home'. Indeed, large

numbers of juveniles have been documented in artisanal fishery landings from different sites in Sonora, Sinaloa, and Baja California Sur (Torres-Huerta 1999), all of which border the GOC. Females may be just as likely to pup at any of these sites within the GOC, rendering them philopatric on a regional scale.

Female philopatry. MtDNA control region diversity among sampled individuals from La Paz Bay is low, with only two haplotypes found previously by Nance et al. (*in prep*), and this has limited the use of maternally inherited markers to detect temporal structure at this site. However, differences across years at La Paz Bay might also result from philopatric females who mate with a different genetic stock of males each season and use of nuclear microsatellites, while more variable than mtDNA, hinder detection of such sex-specific behavior. While our data do not permit us to infer such sex-biased dispersal, it may explain the temporal structure evident in La Paz Bay.

Explanation for Temporal and Spatial Patchiness

As mentioned previously, it has recently been shown that present-day levels of connectivity along the Eastern Pacific (EP) range of *S. lewini* are ecologically low, based on both nuclear and mtDNA, but such low connectivity has developed fairly recently (Nance et al. *in review*). Furthermore, mismatch analyses from this same study indicated that seven *S. lewini* populations between Mexico and Ecuador last experienced a population expansion at roughly the same time (185,000 – 260,000 years ago), suggesting a common, ancient demographic history. These data imply that the spatial genetic

structure evident in the EP today is recent, and that this shark was far more connected by gene flow along this continental margin in the past (Nance et al. *in review*).

If this is true, then *S. lewini* was likely not philopatric in the past, as high historic gene flow across its EP range is inconsistent with site-fidelity to breeding and/or reproductive grounds. As it seems unlikely that philopatric behavior would evolve only recently, the temporal variation within La Paz Bay, which is at times greater than spatial variation between La Paz and Tarcoles (separated by over 3000 km), might reflect the fact that *S. lewini* was neither philopatric in the past, nor is it today. Therefore, current patterns of spatial structure may not be the result of site-fidelity in *S. lewini*, but rather caused by more complicated evolutionary processes. More detailed parentage analyses in which maternity and sibship are known *a priori*, in conjunction with tagging data from breeding adult males and females could help determine conclusively whether site-fidelity exists in *S. lewini*.

Implications for Mating System

Given the propensity of juveniles to remain close to shore until sexually mature, or nearly so (e.g. Klimley 1987; Stevens & Lyle 1989), we expected many of the juveniles sampled in La Paz Bay to have been born there, and potentially from the same litter, either as full- or half-siblings. While we found a high proportion of half-siblings both within and between sampling years in La Paz Bay, whether these half-siblings predominantly share mothers or fathers is unknown, though the fact that mothers must frequent La Paz Bay for parturition suggests that half-sibs are more likely to be litter

mates (maternal sibs) rather than individuals that share the same father, but came from different litters (paternal sibs). This may support the possibility of female philopatry, mentioned above, but also implies polyandry may be prevalent in *S. lewini*.

Polyandry is quite common in the lemon shark, *Negaprion brevirostris*, for whom microsatellite and tagging data revealed that females return to a nursery lagoon at Bimini Island in the Bahamas on a biennial cycle for parturition (Feldheim et al. 2002, 2004). The latter study showed that 86% of litters at Bimini were comprised of maternal half-siblings having different fathers. Polyandrous litters have also been identified in the nurse shark, *Ginglymostoma cirratum* (Ohta et al. 2000; Saville et al. 2002), the bignose shark, *Carcharhinus altimus* (Daly-Engel et al. 2006), the thornback ray, *Raja clavata* L. (Chevolot et al. 2007), and the sandbar shark, *Carcharhinus plumbeus*, though roughly equal frequencies of polyandry and monogamy were found in *C. plumbeus* (Daly-Engel et al. 2007).

Though parentage can not be reliably assigned with the juveniles sampled from La Paz Bay since they were collected from fishermen with no *a priori* knowledge pertaining to littermates (Jones et al. 2010), it is unknown whether *S. lewini* half siblings are maternal or paternal. However, given the frequency of polyandry in sharks and the fact that half-siblings are much more prevalent than full-siblings within and between sampling years, it would seem that monogamy is not common in *S. lewini*. Though little is known about breeding behavior in *S. lewini*, social behavioral displays have been documented among large schools comprised mainly of females in the vicinity of seamounts in the Gulf of California, and it is thought that these schools function to

facilitate such conspecific social interactions (Klimley 1985). It may be that males, and the females with whom they interact in these schools, comprise distinct breeding populations. To test this hypothesis, of course, would require genotyping adults within several of these schools at seamounts across a wide geographic area.

Implications for Conservation

The concern for endangered semi-pelagic sharks like *S. lewini*, with females who frequent protected, coastal zones for parturition, is that such behavior will increase their risk of overfishing. Large, pregnant females become targeted by fishers for their size and proximity to shore (Hueter 1998; NOAA 1999; NMFS 2001) and this can lead to unequal sex ratios in the population, resulting in the number of females being the upper limit to N_e regardless of how many adult, breeding males there are in the population at large (Chapman et al. 2004).

Similarly, the use of distinct breeding and/or nursery areas in philopatric species increases the risk of local extinction due to overfishing. Such areas become a critical component of the reproductive success of populations that use them exclusively, and once these populations are depleted it may take a long time for them to re-establish (Robichaud & Rose 2001; Hueter et al. 2004). Our finding that juveniles in La Paz Bay and the adults producing them year after year, are not from one genetic stock but perhaps from several regional breeding populations may buffer the effects of localized overfishing in the short-term. However, as these populations continue to decline, all the ills associated with small N_e [increased inbreeding, increased risk of local extinction,

lower overall diversity and associated decreased ability to adapt to changes in the environment (Frankham 1995)] will put these populations at risk of extinction unless cooperative, international management strategies are adopted.

Literature Cited

Baker, C. S., L. Florez-Gonzalez, B. Abernethy, H. C. Rosenbaum, R. W. Slade, J. Capella, and J. L. Bannister. 1998. Mitochondrial DNA variation and maternal gene flow among humpback whales of the Southern Hemisphere. Mar. Mammal. Sci. 14:721-737.

Bowen, B. W., A. L. Bass, L. Soares, and R. J. Toonen. 2005. Conservation implications of complex population structure: lessons from the loggerhead turtle (*Caretta caretta*). Mol. Ecol. 14:2389-2402.

Bull, J. T. and R. Shine. 1979. Interoparous animals that skip opportunities for reproduction. Am. Nat. 114:296-303.

Carlsson, J., R. J. McDowell, L. Carlsson, and J. E. Graves. 2007. Genetic identity of YOY bluefin tuna from the eastern and western Atlantic spawning areas. Heredity 98:23-28.

Castro, J. I. 1993. The shark nursery of Bulls Bay, South Carolina, with a review of the shark nurseries of the southeastern coast of the United States. Environ. Biol. Fish. 38:37-48.

Chapman, D. D., P. A. Prodöhl, J. Gelsleichter, C. A. Manire, and M. S. Shivji. 2004. Predominance of genetic monogamy by females in a hammerhead shark, *Sphyrna tiburo*: implications for shark conservation. Molecular Ecology 13: 1965-1974.

Chapman, D. D., D. Pinhal, and M. S. Shivji. 2009. Tracking the fin trade: genetic stock identification in western Atlantic scalloped hammerhead sharks Sphyrna lewini. Endang. Species Res., online Dec. 2009.

Chapuis, M. P., and A. Estoup. 2007. Microsatellite null alleles and estimation of population differentiation. Mol. Biol. Evol. 24:621-631.

Chevolot, M., J. R. Ellis, A. D. Rijnsdorp, W. T. Stam, and J. L. Olsen. 2007. Multiple paternity in the thornback ray *Raja clavata* L. J. Heredity 98:712-715.

Clarke, T. A. 1971. The ecology of the scalloped hammerhead shark, *Sphyrna lewini*, in Hawaii. Pac. Sci. 25:133-144.

Compagno, L. J. V. 1984. FAO Species catalogue. vol. 4, parts 1 & 2, Sharks of the World. FAO Fisheries Synopsis, p. 125.

Daly-Engel, T. S., R. D. Grubbs, K. N. Holland, R. J. Toonen, and B. W. Bowen. 2006. Assessment of multiple paternity in single litters from three species of carcharhinid sharks in Hawaii. Environ. Biol. Fish. 76:419-424.

Daly-Engel, T. S., R. D. Grubbs, B. W. Bowen, and R. J. Toonen. 2007. Frequency of multiple paternity in an unexploited tropical population of sandbar sharks (*Carcharhinus plumbeus*). Can. J. Fish. Aquat. Sci. 64:198-204.

De Bruyn, P., S. F. J. Dudley, G. Cliff, and M. J. Smale. 2005. Sharks caught in the protective gill nets off KwaZulu-Natal, South Africa. 11. The scalloped hammerhead shatk, *Sphyrna lewini* (Griffith and Smith). Afr. J. Mar. Sci. 27:517-528.

Duncan, K. M., A. P. Martin, B. W. Bowen, H. G. De Couet. 2006. Global phylogeography of the scalloped hammerhead shark (*Sphyrna lewini*). Mol. Ecol. 15:2239-2251.

Excoffier, L., G. Laval, and S. Schneider. 2005. Arlequin ver. 3.0: An integrated software package for population genetics data analysis. Evol. Bioinformatics Online 1:47-50.

Feldheim, K. A., S. H. Gruber, and M. V. Ashley. 2002. The breeding biology of lemon sharks at a tropical nursery lagoon. Proc. R. Soc. Biol. Sci., Lond., Ser. B 269:1655-1661.

Feldheim, K. A., S. H. Gruber, and M. V. Ashley. 2004. Reconstruction of the parental microsatellite genotypes reveals female polyandry and philopatry in the lemon shark, *Negaprion brevirostris*. Evolution 58:2332-2342.

Frankham, R. 1995. Conservation genetics. Annu. Rev. Genetics 29:305-327.

Hazin, F., A. Fischer, and M. Broadhurst. 2001. Aspects of reproductive biology of the scalloped hammerhead shark, *Sphyrna lewini*, off northeastern Brazil. Enviro. Biol. Fish. 61:51-159.

Hearn, A., J. Ketchum, P. Klimley, and E. Espinoza. *in prep*. Hotspots within hotspots? Hammerhead shark movements around Wolf Island, Galapagos Marine Reserve.

Hubisz, M. J., D. Falush, M. Stephens, and J. K. Pritchard. 2009. Inferring weak population structure with the assistance of sample group information. Mol. Ecol. Res. 9:1322-1332.

Hueter, R. E. 1998. Philopatry, natal homing and localized stock depletion in sharks. Shark News 12 (Newsletter of the IUCN/SSC Shark Specialist Group) 1-2.

Hueter, R. E., M. R. Huepel, E. J. Heist, and D. B. Keeney. 2004. Evidence of philopatry in sharks and implications for the management of shark fisheries. J. Northw. Atl. Fish. Sci. 35:239-247.

IUCN, Species Survival Commission. 2007. Assessment of global status of pelagic and semi-pelagic sharks and rays. Available at: http://www.iucn.org/media/materials/releases/?103/More-oceanic-sharks-added-to-the-IUCN-Red-List. Released February 2007.

Jones, A. G., C. M. Small, K. A. Paczolt, and N. L. Ratterman. 2010. A practical guide to methods of parentage analysis. Mol. Ecol. Res. 10:6-30.

Keeney, D. B., and E. J. Heist. 2003. Characterization of microsatellite loci isolated from the blacktip shark and their utility in requiem and hammerhead sharks. Mol. Ecol. Notes 3:501-504.

Keeney, D. B., M. Heupel, R. E. Hueter, and E. J. Heist. 2003. Genetic heterogeneity among blacktip shark, *Carcharhinus limbatus*, continental nurseries along the US Atlantic and Gulf of Mexico. Mar. Biol. 143:1039-1046.

Keeney, DB, MR Heupel, RE Hueter, EJ Heist. 2005. Microsatellite and mitrochondrial DNA analyses of the genetic structure of blacktip shark (*Carcharhinus limbatus*) nurseries in the northwestern Atlantic, Gulf of Mexico, and Caribbean Sea. Molecular Ecology 14: 1911- 1923.

Klimley, A. P. 1985. Schooling in Sphyrna lewini, a species with low risk of predation: a non-egalitarian state. Zeitsch. Tierpsych. 70:297-319.

Klimley, A. P. 1987. The determinants of sexual segregation in the scalloped hammerhead shark, *Sphyrna lewini*. Enviro. Biol. .Fish. 18:27-40.

Kohler, N. E., J. G. Casey, and P. A. Turner. 1998. NMFS cooperative shark tagging program, 1962-93: An atlas of shark tag and recapture data. Mar. Fish. Rev. 60:187.

Liu, K. M. and C. T. Chen. 1999. Demographic analysis of the scalloped hammerhead, *Sphyrna lewini*, in the Northwestern Pacific. Fish. Sci. 65:218-223.

Mayr, E. 1963. Animal Species and Evolution. Belknap Press of Harvard University Press, Cambridge, Mass. 797p.

Nance, H. A., T. S. Daly-Engel, and P. B. Marko. 2009. New microsatellite loci for the endangered scalloped hammerhead shark, *Sphyrna lewini*. Mol. Ecol. Res. 9:955-957.

Nance, H. A., A. P. Klimley, F. Galván-Magaña, J. Martínez-Ortíz, and P. B. Marko. *in review*. Decline and divergence: Evolutionary processes causing population structure in the scalloped hammerhead shark, *Sphyrna lewini*, in the Eastern Pacific. Mark D. Rausher, Editor-in-chief, Evolution.

National Marine Fisheries Service (NMFS). 2001. Final United States National Plan of Action for the Conservation and Management of Sharks. National Marine Fisheries Service Report. US Department of Commerce, National Oceanic and Atmospheric Administration, Silver Spring, MD.

National Oceanic and Atmospheric Administration (NOAA). 1999. Essential Fish Habitat Source Document: Spiny Dogfish Life History Characteristics. NOAA Technical Memorandum NMFS-NE-150. Northeast Fisheries Science Center, Woods Hole, MA, USA.

Nei, M. 1977. F-statistics and analysis of gene diversity in subdivided populations. Ann. Hum. Genet. 41:225-233.

Ohta, Y., K. Okamura, E. C. McKinney, S. Bartl, K. Hashimoto, and M. F. Flajnik. 2000. Primitive synteny of vertebrate histocompatibility complex class I and class II genes. Proc. Nat. Acad. Sci., USA 97:4712-4717.

Pardni, AT, CS Jones, LR Noble, B Kreiser, H Malcolm, BD Bruce, JD Stevens, G Cliff, MC Scholl, M Francis, CAJ Duffy, AP Martin. 2001. Sex-biased dispersal in great white sharks. Nature 412: 139-140.

Peakall, R., and P. E. Smouse. 2005. GenAlEx 6: genetic analysis in excel – Population genetic software for teaching and research. Australian National University, Canberra.

Pratt, H. L., and J. C. Carrier. 2001. A review of elasmobranch reproductive behavior with a case study on the nurse shark, *Ginglymostoma cirratum*. Env. Biol. Fish. 60:157-188.

Queller, D. C., and K. F. Goodnight. 1989. Estimating relatedness using genetic markers. Evolution 43: 258-275.

Quinn, T. P., E. C. Volk, and A. P. Hendry. 1999. Natural otolith microstructure patterns reveal precise homing to natal incubatioin sites by sockeye salmon (*Oncorhynchus nerka*). Can. J. Zool. 77:766-775.

Rice, W. R. 1989. Analyzing tables of statistical tests. Evolution 43:223-225.

Rideout, R. M., G. A. Rose, and M. P. M. Burton. 2005. Skipped spawning in female iteroparous fishes. Fish and Fisheries 6:50-72.

Robichaud, D. and G. A. Rose. 2001. Multiyear homing of Atlantic cod to spawning ground. Can. J. Fish. Aquat. Sci. 58:2325-2329.

Saville, K. J., A. M. Lindley, E. G. Maries, J. C. Carrier, and H. L. Pratt. 2002. Multiple paternity in the nurse shark, *Ginglymostoma cirratum*. Env. Biol. Fish. 63:347-351.

Stevens, J. D. and J. M. Lyle. 1989. Biology of three hammerhead shatks (*Eusphyra blochii*, *Sphyrna mokarran*, and *S. lewini*) from Nothern Australia. Aust. J. Freshwater Res. 40:129-146.

Van Oosterhout, C., W. F. Hutchinson, D. P. M. Wills, and P. Shipley. 2004. Micro Checker: software for identifying and correcting genotyping errors in microsatellite data. Mol. Ecol. Notes 4:535-538.

Wang, J. 2004. Sibship reconstruction from genetic data with typing errors. Genetics 166:1963-1979.

Wang, J. 2009. A new method for estimating effective population sizes from a single sample of multilocus genotypes. Mol. Ecol. 18:2148-2164.

Yano, K., F. Sato, and T. Takahashi. 1999. Observations of the mating behavior of the manta ray, *Manta birostris*, and the Ogasawara Islands, Japan. Ichth. Res. 46:289-296.

Zanella, I. 2008. Caracterización de la pesca y algunos aspectos sobre la reproducción, alimentación y distribución del tiburón martillo *Sphyrna lewini*, Sphyrnidae, en el Pacífico de Costa Rica. Tesis de M. en C., Universidad Nacional de Costa Rica, Instituto Internacional de Conservacion y Manejo de Vida Silvestre, 104 pp.

Table 4.1. AMOVA results from global analyses including all sampling years from La Paz (LAP) only, and all La Paz samples plus the Tarcoles (TAR), Costa Rica sample. P-values in bold were significant at $\alpha = 0.05$.

Samples	Source of variation	d.f.	SS	Variance components	% Var.	F_{ST}	p-val
All LAP years	among pops	3	17.549	0.01094 Va	0.21	0.002	0.117
	within pops	210	1106.404	5.26859 Vb	99.79		
All LAP and TAR2007	among pops	4	25.863	0.02033 Va	0.38	0.004	**0.009**
	within pops	289	1527.154	5.28427Vb	99.62		

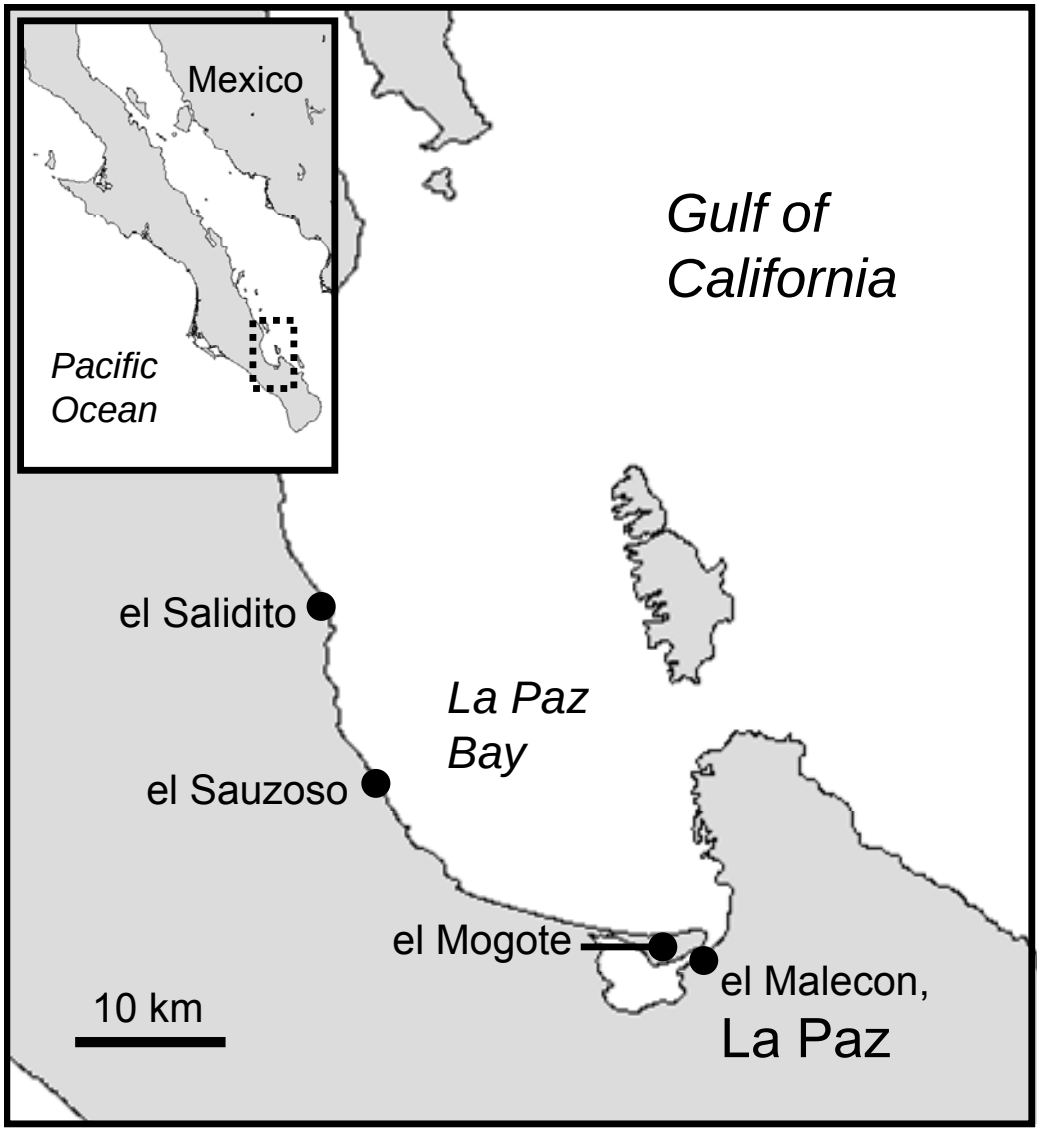
Table 4.2. Pairwise F_{ST} (above diagonal) and associated p-values (below diagonal) characterizing level of genetic differentiation between sampling years in La Paz and Tarcoles. F_{ST} values in bold were significant at $\alpha = 0.05$.

Sample	La Paz 2001	La Paz 2004	La Paz 2006	La Paz 2007	Tarcoles 2007
La Paz 2001	*	0.950	0.640	0.978	0.552
La Paz 2004	-0.006	*	0.000	0.241	0.048
La Paz 2006	-0.002	**0.019**	*	0.018	0.002
La Paz 2007	-0.008	0.002	**0.008**	*	0.012
Tarcoles 2007	-0.001	**0.006**	**0.010**	**0.008**	*

Table 4.3. Percent of all individuals (n) in sample years (individually and combined) that were members of full- and/or half-sibling pairs. Half-sib and full-sib pairs had relatedness coefficients ≥ 0.25 and 0.50, respectively in analyses using Genalex and Queller and Goodnight's (1989) relatedness coefficient, r. Half-sib and full-sib pairs inferred using COLONY2 (Wang 2009), had likelihoods of ≥ 95%.

		Queller & Goodnight's r		Colony2	
Year	n	Full sib	Half sib	Full sib	Half sib
2001	31	0%	48.39%	6.45%	74.19%
2004	22	0%	59.09%	0%	36.36%
2006	30	0%	53.33%	0%	60.00%
2007	24	8.33%	70.83%	0%	50.00%
All Years	107	8.41%	81.31%	10.28%	85.05%

Figure 1. Map showing La Paz Bay, where all juvenile *S. lewini* were caught by artisanal fishermen, and the four fish camps near La Paz where samples were taken.



APPENDICES

Appendix A

Microsatellite Statistics per Locus, per Population

	LAP2006					LAP2007				MAZ2006				MAZ2007				TAR2007		
Locus	***At***	***Ap***	***Ho***	***He***	***p-val***	***Ap***	***Ho***	***He***	***p-val***	***Ap***	***Ho***	***He***	***p-val***	***Ap***	***Ho***	***He***	***p-val***	***Ap***	***Ho***	***He***
Cli12	37	0	0.680	0.882	0.009	0	0.870	0.918	0.177	0	0.941	0.952	0.237	0	1.000	0.946	0.877	0	0.914	0.944
Cli100	13	0	0.929	0.810	0.220	0	0.792	0.809	0.619	0	0.824	0.836	0.675	0	0.868	0.834	0.724	0	0.794	0.854
SLE013	29	1	0.700	0.667	1.000	2	0.700	0.753	0.544	2	0.762	0.698	0.765	2	0.737	0.631	0.529	0	0.641	0.648
SLE025	46	0	0.933	0.916	0.747	0	0.778	0.884	0.108	1	0.857	0.861	0.199	3	0.946	0.891	0.788	2	0.889	0.848
SLE027	27	0	0.800	0.694	0.494	0	0.696	0.754	0.484	0	0.762	0.726	0.252	0	0.639	0.649	0.694	0	0.725	0.652
SLE028	17	0	0.750	0.828	0.410	0	0.909	0.832	0.223	0	0.762	0.813	0.656	1	0.694	0.838	0.202	0	0.750	0.841
SLE033	15	1	0.926	0.798	0.799	0	0.762	0.741	0.047	0	0.750	0.819	0.039	0	0.778	0.786	0.067	0	0.788	0.766
SLE038	16	1	0.767	0.849	0.667	0	0.800	0.854	0.616	0	0.619	0.705	0.315	1	0.667	0.746	0.799	0	0.795	0.751
SLE045	7	0	0.552	0.657	0.099	0	0.565	0.677	0.242	0	0.476	0.619	0.216	0	0.583	0.699	0.107	0	0.650	0.747
SLE053	22	1	0.767	0.871	0.038	0	0.435	0.704	0.006	1	0.850	0.815	0.596	0	0.639	0.790	**0.001**	0	0.775	0.843
SLE071	21	1	0.679	0.828	0.003	0	0.773	0.722	0.191	1	0.800	0.638	0.380	0	0.737	0.747	0.024	1	0.615	0.754
SLE077	47	1	0.963	0.892	0.836	0	0.864	0.918	0.623	0	0.947	0.910	0.441	2	0.895	0.882	0.367	3	0.949	0.894
SLE081	13	0	0.857	0.797	0.987	0	0.818	0.814	0.174	0	0.700	0.769	0.770	0	0.865	0.831	0.519	0	0.763	0.797
SLE086	10	0	0.667	0.727	0.279	0	0.773	0.742	0.499	0	0.550	0.665	0.188	0	0.730	0.732	0.683	0	0.600	0.627
SLE089	13	0	0.926	0.864	0.512	0	0.773	0.816	0.609	0	0.950	0.847	0.919	0	0.868	0.829	0.303	0	0.769	0.828

	TAR2008				SCA0708				CEB2008				GPA2008				MAN0708			
Locus	***Ap***	***Ho***	***He***	***p-val***	***Ap***	***Ho***	***He***	***p-val***	***Ap***	***Ho***	***He***	***p-val***	***Ap***	***Ho***	***He***	***p-val***	***Ap***	***Ho***	***He***
Cli12	1	0.923	0.943	0.619	2	0.871	0.940	0.071	0	0.810	0.944	0.044	0	0.667	0.954	**0.000**	1	0.940	0.249
Cli100	0	0.825	0.811	0.465	2	0.767	0.806	0.230	0	0.619	0.796	0.067	0	0.778	0.810	0.980	0	0.853	0.833
SLE013	2	0.650	0.742	0.079	1	0.611	0.662	0.468	0	0.714	0.632	0.642	0	0.556	0.739	0.775	5	0.583	0.645
SLE025	0	0.825	0.881	0.155	6	0.841	0.875	0.388	1	0.895	0.876	0.985	0	1.000	0.909	0.775	3	0.813	0.870
SLE027	0	0.700	0.737	0.015	2	0.539	0.594	0.041	0	0.619	0.659	0.016	1	0.750	0.767	0.845	1	0.671	0.658
SLE028	1	0.950	0.834	0.031	1	0.852	0.842	0.264	0	0.857	0.840	0.854	0	0.889	0.843	0.916	0	0.824	0.810
SLE033	1	0.795	0.821	0.343	3	0.779	0.801	0.010	0	0.667	0.756	0.400	0	0.889	0.765	0.832	0	0.735	0.763
SLE038	0	0.700	0.800	0.399	1	0.764	0.823	0.022	0	0.762	0.829	0.921	0	1.000	0.869	0.764	1	0.770	0.784
SLE045	0	0.564	0.670	0.025	2	0.583	0.671	0.024	0	0.762	0.689	0.735	0	0.444	0.752	0.127	0	0.662	0.693
SLE053	0	0.725	0.832	0.320	1	0.727	0.872	**0.000**	0	0.800	0.867	0.725	0	0.889	0.928	0.718	1	0.803	0.888
SLE071	0	0.675	0.708	0.716	0	0.554	0.673	**0.002**	0	0.789	0.794	0.740	0	0.667	0.667	0.677	2	0.675	0.709
SLE077	3	0.947	0.917	0.811	4	0.820	0.868	0.138	0	0.895	0.909	0.538	0	0.889	0.902	0.864	5	0.909	0.892
SLE081	0	0.800	0.818	0.967	0	0.875	0.805	0.842	1	0.947	0.819	0.567	0	0.667	0.837	0.722	1	0.800	0.813
SLE086	1	0.795	0.680	0.028	0	0.631	0.749	**0.000**	0	0.947	0.787	0.550	0	0.667	0.725	0.088	0	0.707	0.759
SLE089	0	0.900	0.842	0.269	0	0.822	0.844	0.137	1	0.895	0.885	0.758	0	0.778	0.850	0.845	0	0.808	0.839

Figure A-1: Microsatellite statistics per locus, per population. At = total number of alleles per locus across all populations. Ap = private alleles per locus, per population. Ho = observed heterozygosity per locus, per population, and He = expected heterozygosity per locus, per population, as calculated in Arlequin v. 3.11 (Excoffier et al. 2005). P-values in bold were significant after sequential Bonferroni correction of alpha (α).

.Appendix B

Microsatellite Statistics per Locus, per Sample

	LAP2001			LAP2004			LAP2006			LAP2007			TAR2007	
Locus	***Ho***	***He***	***p-val***	***Ho***	***He***	***p-val***	***Ho***	***He***	***p-val***	***Ho***	***He***	***p-val***	***Ho***	***He***
Cli12	0.774	0.936	0.046	0.091	0.921	0.652	0.680	0.882	0.009	0.870	0.918	0.177	0.914	0.944
Cli100	0.846	0.799	0.181	0.091	0.818	0.691	0.929	0.810	0.220	0.792	0.809	0.619	0.794	0.854
SLE013	0.645	0.593	0.525	0.409	0.537	0.009	0.700	0.667	1.000	0.700	0.753	0.544	0.641	0.648
SLE025	0.893	0.925	0.125	0.909	0.860	0.860	0.933	0.916	0.747	0.778	0.884	0.108	0.889	0.848
SLE027	0.677	0.880	0.011	0.955	0.863	0.971	0.800	0.694	0.494	0.696	0.754	0.484	0.725	0.652
SLE028	0.677	0.765	0.082	0.591	0.737	0.025	0.750	0.828	0.410	0.909	0.832	0.223	0.750	0.841
SLE033	0.774	0.790	0.683	0.773	0.752	0.577	0.926	0.798	0.799	0.762	0.741	0.047	0.788	0.766
SLE038	0.652	0.644	0.318	0.545	0.759	0.298	0.767	0.849	0.667	0.800	0.854	0.616	0.795	0.751
SLE045	0.536	0.721	0.011	0.619	0.609	1.000	0.552	0.657	0.099	0.565	0.677	0.242	0.650	0.747
SLE053	0.833	0.779	0.204	0.857	0.803	0.359	0.767	0.871	0.038	0.435	0.704	**0.006***	0.775	0.843
SLE071	0.806	0.740	0.715	0.600	0.687	0.633	0.679	0.828	0.003	0.773	0.722	0.191	0.615	0.754
SLE077	0.742	0.913	0.016	0.900	0.885	0.469	0.963	0.892	0.836	0.864	0.918	0.623	0.949	0.894
SLE081	0.900	0.833	0.592	0.857	0.801	0.472	0.857	0.797	0.987	0.818	0.814	0.174	0.763	0.797
SLE086	0.645	0.777	0.040	0.714	0.734	0.544	0.667	0.727	0.279	0.773	0.742	0.499	0.600	0.627
SLE089	0.867	0.802	0.451	0.864	0.862	0.927	0.926	0.864	0.512	0.773	0.816	0.609	0.769	0.828

Figure B-1: Microsatellite statistics per locus, per sample. LAP refers to samples collected in La Paz Bay while TAR refers to the sample from Tarcoles, Costa Rica. Ho = observed heterozygosity per locus, per sample, and He = expected heterozygosity per locus, per sample, as calculated in Arlequin v. 3.11 (Excoffier et al. 2005). P-values with an asterisk (*) were significant after sequential Bonferroni correction of alpha (α).

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